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The impact of dietary manipulation of rumen pH on health and productivity in dairy cows

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Contents

To my parents: Rodolfo y María Elena

To Roy Fawcett

Declaration

I hereby declare that I have composed the present thesis. This is my own work and any assistance received has been duly acknowledged. Furthermore publications that arose from this work are my own work. The work described and the results presented herein have not been submitted for any other degree or professional qualification.

Virgilio Ambriz Vilchis

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Commonly used abbreviations

BCS: Body condition score

BW: Body weight

CHO: Carbohydrate

CP: Crude protein

d: Days

DIM: Days in milk

DM: Dry matter

DMI: Dry matter intake

FCM: Fat corrected milk

h: Hour

min: Minutes

NDF: Neutral detergent fibre

PMR: Partial mixed ration

SARA: Subacute ruminal acidosis

uNDF: Un-degradable Neutral detergent fibre

VFA: Volatile fatty acid

Abstract

Current feeding strategies for dairy cows focus on meeting the energy requirements for high levels of milk production. However a major concern is the effect that these feeding regimes might have on rumen pH, which can have harmful effects on the cow and rumen microbial population. Several interventions have been used to counteract the effects of low rumen pH such as the use of probiotics e.g. yeast (*Saccharomyces cerevisiae*). However benefits have been inconclusive due to large individual animal variation in responses to treatment observed. The use of novel monitoring technologies can help assess the effect that different dietary interventions have on performance, rumen pH and rumen health. Data from three on-farm dairy cow trials (Trial 1 standard diet plus yeast; Trial 2 standard diet plus acidotic challenge plus yeast; Trial 3 cows grazing grass plus yeast) was used to evaluate the use of rumination collars (RC), rumen pH boluses, a whole cow dynamic mechanistic simulation model (SM) and the effect that different feeding strategies have on performance rumen pH dynamics and rumination time.

No statistically significant differences between Control (no yeast) and Treatment (addition of yeast) diets were observed on any of the parameters measured. The lack of animal response to yeast supplementation observed in the three feeding Trials could be attributed to the stage of lactation, as the cows were passed peak lactation.

Comparison of rumination time obtained with the RC and visual observations (obtained directly and from video recordings) suggest that the RC can be used to determine rumination time in housed cows. However its poor performance in grazing environments makes its use not advisable in cows outside at grass.

The rumen pH boluses provided detailed and accurate data on circadian rumen pH. Highly varied individual responses to the feeding strategies were observed. This resulted in a diverse degree of risk of individual cows which experienced sub-acute rumen acidosis.

The SM was able to accurately predict circadian pH, compared against the data obtained from Trials 1 and 2. The model provided pH values that were in agreement with those obtained with the rumen boluses. The use of new technologies to monitor cows individually could aid in whole-herd management, for example by setting thresholds for rumen pH and rumination time related to individual cow status, and then trigger appropriate interventions.

Publications

Research articles (peer-reviewed)

Ambriz-Vilchis V., N.S. Jessop, R.H. Fawcett, D.J. Shaw, A.I. Macrae. (2015) Comparison of rumination activity measured using rumination collars against direct visual observations and analysis of video recordings of dairy cows in commercial farm environments. Journal of Dairy Science 98, Issue 3: 1750 – 1758.

Book chapters (peer-reviewed)

Ambriz-Vilchis V., R.H. Fawcett, D.J. Shaw, A.I. Macrae and N.S. Jessop. (2015) 8.2. Biopara-Milk: a whole cow simulation model for the prediction of rumen pH. Pages 299 – 306 in Precision Livestock Farming Applications. Wageningen Academic Publishers

Conference abstracts

Ambriz-Vilchis V. Effect of dietary yeast supplementation on production parameters, rumen pH and rumination time of dairy cows in commercial farm environments. Annual Royal (Dick) School of Veterinary Studies Research Student day, 30th April 2015 (oral).

Ambriz-Vilchis V., R.H. Fawcett, D.J. Shaw, A.I. Macrae and N.S. Jessop. Biopara-Milk: a whole cow simulation model: rumen pH predictions. 8th International Workshop Modelling Nutrient Digestion and Utilization in farm animals Cairns, Australia 16th September 2014 (oral and poster)

Ambriz-Vilchis V., N.S. Jessop, R.H. Fawcett, D.J. Shaw, A.I. Macrae. Comparison of rumination time obtained with rumination collars against direct visual observations in cubicle housed and grazing dairy cows. Joint International Symposium on the Nutrition of Herbivores/ International Symposium on Ruminant Physiology (ISNH/ISRP) Canberra, Australia 8th to 12th September 2014 (Poster)

Ambriz-Vilchis V., N.S. Jessop, R.H. Fawcett, D.J. Shaw, A.I. Macrae. Effect of dietary yeast supplementation on production, rumen pH and rumination time of cubicle-housed dairy cows with induced episodes of sub-acute rumen acidosis. Joint International Symposium on the Nutrition of Herbivores/ International Symposium on Ruminant Physiology (ISNH/ISRP) Canberra, Australia 8th to 12th September 2014 (Poster)

Ambriz-Vilchis V., R.H. Fawcett, D.J. Shaw, A.I. Macrae and N.S. Jessop. Biopara-Milk: a whole cow simulation model for the prediction of rumen pH. 65th Annual Meeting of the European Federation of Animal Science (EAAP) Copenhagen, Denmark 25th August 2014 (oral)

Ambriz-Vilchis V. Assessment of rumination activity in dairy cows using rumination collars and behavioural analyses. Annual Royal (Dick) School of Veterinary Studies Research Student day, 24th April 2014 (poster).

Ambriz-Vilchis V. N.S. Jessop, R.H. Fawcett, D.J. Shaw, A.I. Macrae. Comparison of rumen pH predictions by Biopara-Milk vs. intra ruminal boluses in commercial dairy cows. Congress British Cattle Veterinary Association, 17th – 19th October 2013, Harrogate UK (poster).

Ambriz-Vilchis V. N.S. Jessop, R.H. Fawcett, D.J. Shaw, A.I. Macrae. Comparison of rumen pH predictions by Biopara-Milk vs. intra ruminal boluses in commercial dairy cows. Conference: Does big mean bad? The science behind large scale production 23rd – 24th May 2013, Edinburgh UK (Oral).

Ambriz-Vilchis V. Comparison of video recordings and rumination collars for measuring rumination activity in cubicle housed commercial dairy cows. Annual Royal (Dick) School of Veterinary Studies Research Student day, 24th April 2013 (poster).

Ambriz-Vilchis V. N.S. Jessop, R.H. Fawcett, A.I. Macrae. Comparison of video recordings and rumination collars for measuring activity in cubicle housed commercial dairy cows. Page 4 Advances in Animal Biosciences Proceeding of the British Society of Animal Science. Annual Conference, 16th -17th April 2013 (oral).

Ambriz-Vilchis V. Effect of dietary yeast supplementation on ruminal pH and feeding behaviour of dairy cows. Royal (Dick) School of Veterinary Studies Research Day, 18th April 2012 (poster).

Chapter 1 Introduction

1.1 Dairy production in the UK

The dairy industry is a significant contributor to the UK economy. In 2013 alone it accounted for in excess of £4 billion at market prices which represents 16% of total agricultural output, and it places the UK as the third largest producer of milk within the EU and the tenth worldwide (DEFRA, 2014). This gives the UK dairy industry a strong position to develop, explore export opportunities, and be a key player and contributor to global food security. UK dairy farming is undergoing a constant and sustained process of restructuring. Firstly the number of dairy farms in the UK has been declining (at an average rate of 4% over the past ten years). Secondly the number of animals in the national herd has been falling (from 2.3 million head in the year 2000 to 1.8 million cows in 2014), and lastly the average farm size and milk yield per cow has been rising (Figure 1.1 and 1.2) (DEFRA, 2014).

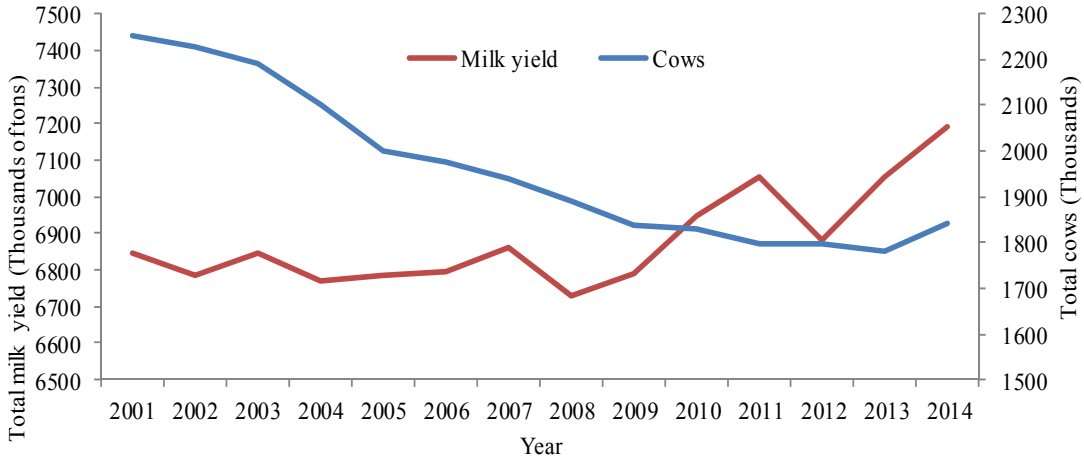

Figure 1.1 Total UK dairy herd and total UK milk production with data from DEFRA (2014).

This concentration of more cattle on fewer farms requires a higher degree of technical ability, monitoring and management of the dairy cow with an improvement in nutrition and feeding.

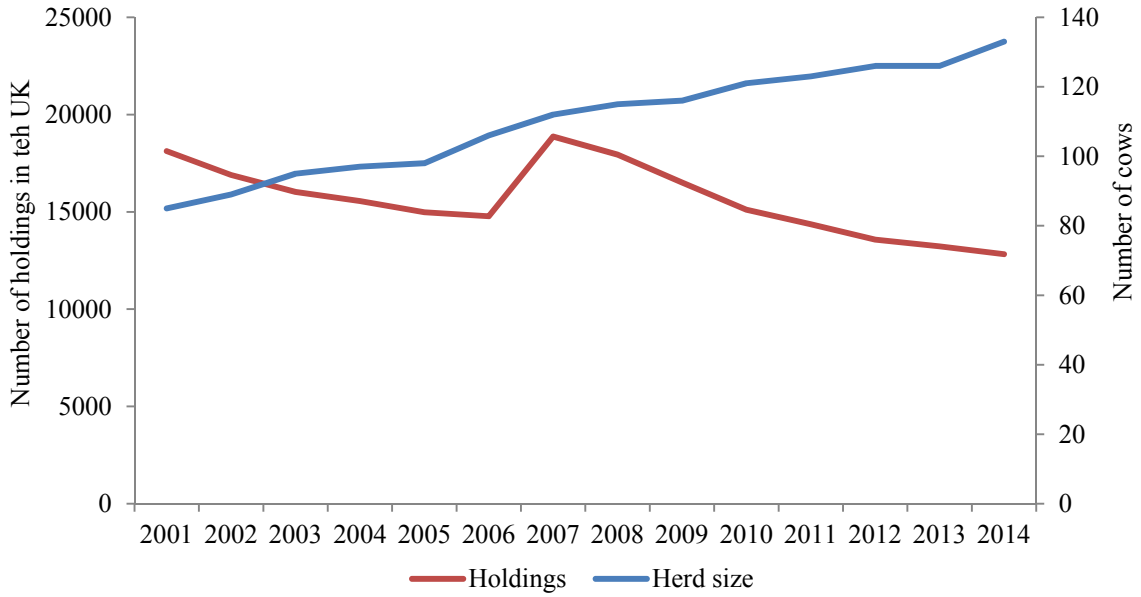

Figure 1.2 Historical average herd size and number of holdings in the UK with data from DEFRA (2014).

There are many different dairy management systems in the UK. However they are mostly based on systems whereby animals graze forage (grass) throughout the months of April to September, with the addition of some conserved forage and concentrate feed as supplement, and during the remaining months a partial mixed ration (PMR) with concentrate feed will be fed to cows housed indoors (Bell et al., 2015). Information published by March et al. (2014) utilising survey data helped in shedding some light into the management regimes used in the UK dairy industry. The authors' findings showed not only the same basic components of the system described above, but also animals housed all year round, and in a smaller proportion animals grazing all year round (March et al., 2014) (Figure 1.3). This tendency for larger herds kept indoors for longer periods of time means that closer monitoring is needed to maintain cow performance and health.

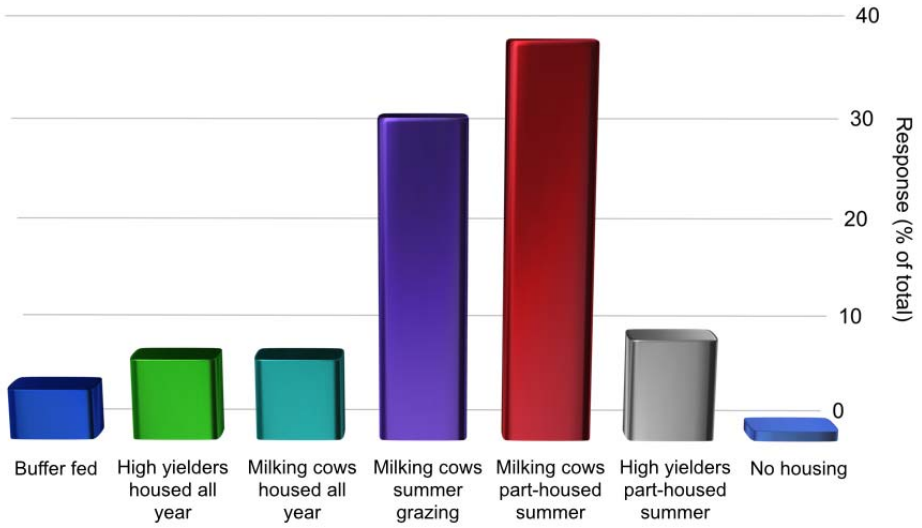

Figure 1.3 Different dairy management systems in the UK, with survey data and presented as percentage of total responses obtained (from (March et al., 2014)).

Dairy farmers and managers in the UK face major challenges in maintaining the health and productivity of their dairy cows. Despite improvements in dairy management, purchased feeds are still the largest variable cost on most dairy farms, even in those farms which rely on large amounts of home-grown feeds. When forage costs are taken into account, the overall feed costs represent at least 50% of a dairy unit's variable costs, and over 25% of total costs (DairyCo, 2013). Therefore feeding management offers the greatest potential for improving profitability on the dairy enterprise. Furthermore when compared to breeding, fertility or other key aspects of herd management which have a longer term impact, the financial effects of changes in feeding are generally apparent within a relatively short space of time. As well as immediate economic effects, feeding adjustments can have a profound effect on labour requirements, machinery and other overhead costs, herd health and fertility and environmental impact. In conclusion, relatively small changes in the efficiency of dairy cow feeding can have a major effect on herd profitability.

1.2 Effects of feeding strategies on health, behaviour and welfare, and performance.

Food intake is the most important factor determining animal performance (Illius and Jessop, 1996). Modern feeding strategies focus on meeting the energy requirements for high levels of milk production by encouraging maximum intake of energy dense, low fibre diets with highly fermentable carbohydrates (Marden et al., 2008). Despite this management regime having a positive effect on milk yield, there are potentially many long term negative effects on dairy cow fertility, welfare and production due to poor rumen health such as reduced feed intake, loss of BCS and increased lameness (Zebeli et al., 2007). More recently, there has been an increased emphasis on the effect of diet on greenhouse gas emissions and concerns regarding the carbon footprint of the dairy industry (Connor et al., 2012). Research suggested that feeding higher levels of concentrate produces less methane, therefore reducing the environmental cost of dairy production. Furthermore feeding highly fermentable diets provides energy precursors needed for higher levels of milk production; however it might increase the risk of health problems including subacute ruminal acidosis (SARA).

1.2.1 Effects of feeding strategies on rumen environment

The rumen is a large fermentation chamber containing a complex microbial ecosystem that works in a dynamic, symbiotic relationship with the host to convert feed into energy and protein (Lean et al., 2014). Therefore the objective of feeding the dairy cow should aim to provide a rumen environment that maximises microbial growth and production without negative effects to the host. In the rumen, feed digestion involves numerous and complex interactions between microbial anaerobic communities (bacteria, fungi, protozoa) and several factors (diet, feed intake level, environment, stress, etc.) which may affect the microbial ecosystem. Rumen microbiota requires a stable, warm, oxygen-free environment for optimum growth. This type of environment is naturally maintained within the rumen at a temperature

range of 37.8 to 42.2°C and to support and favour fibrolytic bacteria, rumen pH between 6.0 and 6.4.

Rumen pH level has a substantial effect on rumen ecology, and alterations to these levels can trigger systemic changes that in turn could affect the host. Low rumen pH (below 6.0) will increase the maintenance requirements of the rumen microbial population and affect reproduction of fibrolytic microbiota. Lower levels of pH can cause illness in the host by altering ruminal motility, inducing rumenitis, affecting rumen papillae and causing hyperkeratosis (Nocek, 1997).

Low rumen pH can cause acidosis by different degrees. Rumen acidosis in dairy cattle is characterised by abnormally low rumen pH values (<5.5 – 5.8), and is generally caused by excess consumption of non-structural carbohydrates (NSC), or decreased consumption of effective fibre. The consumption of NSC increases production of volatile fatty acids (VFA) at a higher rate (Oetzel and Krause, 2006). Rumen acidosis can also be caused when lower forage diets are consumed at a faster rate. This will decrease rumination (DeVries et al., 2007), resulting in reduced saliva outflow and the buffering properties of saliva cannot tackle the increasing amount of VFA which leads to a drop in rumen pH.

In many dairy operations, the challenge is not acute acidosis, but sub-acute rumen acidosis (SARA). There is no consensus as to a precise definition of SARA (Plaizier et al., 2008). However it has been described as a reduction in rumen pH below a threshold of 5.5 (Duffield et al., 2004; Garrett et al., 1999), 5.8 (Beauchemin et al., 2003) or 6.2 (Sauvant et al., 1999) for prolonged periods of time. As clinical signs and diagnosis are complicated, faeces with high content of long fibre particles, diarrhoea and milk butterfat depression are used as proxy measures for the diagnosis of SARA. Garrett et al. (1999) proposed a method to evaluate the incidence of SARA in dairy farms, from rumen samples collected via rumenocentesis 3-4 hours after feeding. A group of cows will be defined as suffering from SARA when 4 or more cows in a sample of 12 presents with a rumen pH <5.5. Animals with pH values between 5.5 and 5.8 are considered borderline, and animals with rumen pH higher than 5.8 are negative for SARA.

It is estimated that the incidence of SARA in dairy herds is between 10 and 30% (Plaizier et al., 2008), a situation that places an extra cost on milk production due to health and performance issues related to this condition. According to Enemark (2008) with data from the US, it is estimated that the economic cost associated with SARA could be in excess of US $ 1000 million per year.

Extensive reviews on SARA have been written (Calsamiglia et al., 2012; Enemark, 2008; Gonzalez et al., 2012; Hook et al., 2011; Kleen and Cannizzo, 2012; Krause and Oetzel, 2006; Plaizier et al., 2008; Plaizier et al., 2012) from which it is concluded that SARA is a major concern to the dairy industry in terms of both reduced productivity and animal welfare. Reviews of the literature by Enemark (2008), Calsamiglia et al. (2012) and Plaizier et al. (2008) provide a thorough description of SARA including definition, causes, effects on performance and health (milk yield, butterfat, feed intake, fibre digestion) and different treatments related to its occurrence in dairy herds. The review of Calsamiglia et al. (2012) focused on the ways of controlling rumen pH, from giving a thorough explanation of the fermentation process involved in the ruminant forestomach to methods of controlling lactic acid production, and feed supplementation for the prevention of SARA.

As it is difficult to diagnose SARA, only a few papers have focused on the incidence and prevalence on-farm. Kleen et al. (2009) investigated the prevalence of SARA on a region of the Netherlands. The authors sampled more than 190 animals from 18 different herds. Using a cut-off point for SARA when rumen pH was lower than 5.5, the authors reported that 13.8 % of the sampled animals had SARA. The prevalence of SARA amongst herds varied from 0 to 38%. Similar results were observed in early lactation high producing dairy cows in Italian herds (Morgante et al., 2007).

SARA is harmful to ruminal cellulolytic bacteria and therefore is detrimental to fibre digestibility. As a result, dairy cattle with SARA are less productive because of reduced feed efficiency, feed digestibility, protein synthesis, milk fat and inconsistent or changes in dry matter intake (DMI) (Krause and Oetzel, 2004).

However precise clinical signs are not well established. SARA has been linked to an increased incidence of diarrhoea, ruminal ulcers, parakeratosis, liver abscess, and laminitis (Dijkstra et al., 2012; Krause and Oetzel, 2004; Li et al., 2009). One of the major concerns with SARA are the diagnostic challenges it presents due to firstly the lack of pathognomonic signs, secondly the problems in obtaining representative rumen fluid samples from which determine rumen pH, and lastly the normal daily fluctuations in rumen metabolism (circadian pH dynamics) which makes interpretation of single time point rumen pH measurements problematic. Due to the difficulties in the diagnosis of SARA, most researchers acknowledge that it is poorly detected in dairy herds (Desnoyers et al., 2009a; Desnoyers et al., 2009b; Kleen and Cannizzo, 2012; O'Grady et al., 2008).

1.2.1.1 Diagnosis of SARA

As stated before due to the lack of pathognomonic signs and subtle clinical changes in individual animals, diagnosis of SARA is difficult and the focus has been placed on herd examination. Rumen samples from 12 animals collected via rumenocentesis 3-4 hours after feeding can be used to sample a herd or group of cows. The group or herd will be defined as suffering from SARA when 4 or more cows present rumen pH <5.5. Animals with pH values between 5.5 and 5.8 are considered borderline and animals with rumen pH higher than 5.8 are negative (Garrett et al., 1999).

Some other empirical or practical on-farm proxy measurements have been described to be useful as aids for the diagnosis of SARA: methods include dung consistency (a high percentage of the animals in the herd with diarrhoea), ration characteristics (highly fermentable diets (e.g. NSC >36% or NDF <32%), feeding behaviour and percentage of cows ruminating whilst at rest (should be >50%), sorting behaviour of a TMR and changes in DMI (Beauchemin and Penner, 2009).

Despite these diagnostic aids, current opinion is that SARA is under-diagnosed because measurement of ruminal pH for definite diagnosis is complicated. Most field techniques performed to obtain rumen pH data are invasive (using rumenocentesis,

rumen fistula, oro-gastric tube), expensive, technically challenging, can be performed only by trained personal and with approval of ethical committees or can only be performed in research animals or in research institutions (Mottram et al., 2008; Richter et al., 2010; Sato et al., 2012b). Moreover when data is obtained from single rumen fluid samples, this data does not give a clear accurate representation of what is otherwise a very dynamic system by not being able to record the normal daily fluctuations in rumen pH.

1.2.1.2 Rumen pH measurement and / or rumen fluid collection techniques.

The objective of all techniques is to collect rumen fluid and analyse it to assess the function and activity of the ruminant forestomach system, and to aid in the diagnosis of diseases specifically SARA. Duffield et al. (2004) presented a review of the different techniques used to obtain rumen fluid and / or measure rumen pH, and the authors describe in detail the techniques and present positive and negative aspects of each of it. A brief description of the methods is presented as follows:

a) Rumen fistula or cannulation: using a modified surgical technique, a plastic cannula is implanted on the left side of the cow's abdomen. The left paralumbar fossa is prepared for aseptic surgery. The site of the incision is chosen so that the flange of the cannula does not impinge on the transverse processes of the lumbar vertebrae, the tuber coxae and/or the last rib. An incision through the skin and different muscles (oblique, transversus, peritoneum, etc.) is performed; all blood vessels are ligated for haemostasis. After four to six days of the procedure and following inspection, the cannula is placed in the fistula (Laflin and Gnad, 2008). The advantages of this procedure are the ease to collect repeated samples of rumen fluid, and the ability to collect several samples throughout the day. However it is the most invasive of all the methods described: it can only be performed on research facilities, and involves ethical concerns due to the surgical procedure involved.

b) Oro-gastric tube (stomach tube, oro-rumen tube, oral stomach tube): a plastic tube is inserted through the mouth, down the oesophagus and into the rumen. A syringe is then used to collect the rumen fluid sample. The initial fluid is discarded because it often contains saliva contamination, which may affect the pH level. The advantage of this procedure is that it is not that invasive, and it is relatively easy to perform. However serious concerns have been raised as to whether it is possible or not to collect rumen fluid without contamination. Results reported by Enemark et al. (2004) showed no relationship ($R^2 = 0.11$) between rumen pH obtained via rumenocentesis or using oro-stomach tube.

c) Rumenocentesis – percutaneous needle aspiration: the ventral abdomen caudal to the xiphoid process and left of the ventral midline is clipped and surgically prepared. A stainless steel needle (14 – 16 gauge needle) is inserted through the skin into the rumen. Using a syringe attached to the inserted needle, rumen fluid is aspired (Nordlund and Garrett, 1994). Rumenocentesis is considered the "gold standard" for rumen fluid collection. The advantages of the procedure is that it is easy to perform and relatively less invasive. However minor effects could be observed in animals after performing rumenocentesis (for example infection, bleeding), and can only be performed by a veterinarian or technician.

d) Indwelling pH meter in fistulated animals: A pH sensor or electrode is placed in the ventral sac of the rumen via the rumen fistula, and suspended in the ventral sac of the rumen. This method has the advantage of providing continuous measurements for several days. However as its use requires fistulated animals, this method is confined to the realm of research and higher education institutions.

e) Automatic pH meter used in rumen fistulated and/or non-fistulated animals: rumen pH boluses. In the last decade, several devices have been developed to measure rumen pH (Table 1.1). The aim of such tools should be to provide detailed and reliable information without compromising either the health or performance of the animal under study. By obtaining continuous, reliable, detailed information of

rumen pH the dynamics of the fermentation process responsible for the circadian pH can be better understood (Krizova et al., 2011).

Table 1.1 Rumen pH studies, comparing measurements obtained with pH meters and rumen bolus.

Reference	Treatment	n	Animals	Fistulated	Mean pH of Treatment (1)	(2)	r	P
Phillips et al. (2010)	(1) rumen bolus (2) Manual Sampling	4	*Bos indicus* cross	Yes	7.03 ± 0.54 (S.D.)	6.64 ± 0.67 (S.D.)	0.93	<0.01
Marden et al. (2005)	(1) Continuous pumping of rumen fluid (2) Manual sampling	2	Dairy cows	Yes	6.52 ± 0.11 (S.E.)	6.65 ± 0.15 (S.E.)	NA	NA
Mottram et al. (2008)[1]	(1) Rumen bolus (2) Manual sampling	4	Beef steers	Yes	Difference of ± 0.2 pH units		NA	NA
Sato et al. (2012a)	(1) Rumen bolus (2) Manual sampling	4	Dairy cows	Yes	6.22 ± 0.54 (S.D.)	6.36 ± 0.55 (S.D.)	0.99	<0.01

[1]Mean pH of treatment not reported, NA = no values reported.

Using the wireless probe reported first by Richter et al. (2010), Krizova et al. (2011) were able to monitor rumen pH in fistulated cows for four days. Their findings showed a highly dynamic pH variation across time, and the influence diet consumption has on rumen environment. The advantage of wireless probes is that they allow continuous measurement of rumen pH values every 15 minutes. However in the aforementioned study, the disadvantage was that it was carried out using fistulated animals, required close technical monitoring and was only able to measure rumen pH dynamics for a few days. Mottram et al. (2008) developed an intra-rumen bolus capable of measuring pH continuously, storing the data and then transmitting it

via radio signal to a remote receiver. Using this device, Phillips et al. (2010) reported a strong correlation between rumen pH recorded manually from rumen samples taken from fistulated animals, and pH measured with the rumen boluses.

The rumen boluses present many advantages including ease of administration (orally administered to the cow) as well as continuously recording and storage of large amounts of data. Some disadvantages include costs, malfunctions that cannot be repaired once deployed, and the potential that the bolus might be expelled by the cow (either by regurgitation or passage).

1.2.2 Rumination as a proxy measure of rumen health

Ruminants occupy an advantageous niche in the animal kingdom. Due to their digestive adaptations, ruminants are capable of converting fibrous, cellulose-rich plant material to energy sources (Van Wieren S.E., 1996). These fibrous materials are firstly subject to pre-gastric fermentation, secondly regurgitated at frequent intervals, re-chewed and finally swallowed back for further degradation. Rumination reduces particle size of feedstuffs for rumen degradation, and initiates the process of extracting soluble contents from the feed. By reducing particle size, rumination increases the passage rate of undigested material from the rumen to the lower digestive tract (Van Soest, 1994). Furthermore by stimulating saliva production, rumination aids in maintaining correct rumen function by keeping rumen pH within suitable levels for microbial cellulolytic activity due to the secretion of bicarbonate in the saliva (Beauchemin et al., 1989).

A combination of factors influence rumination including: nutritional (physical and chemical characteristics of the food material), environmental stressors and day length. For example on the one hand, rations with fibrous feeds increase rumination. On the other hand, high concentrate rations reduce rumination which could lead to rumen acidosis (Dijkstra et al., 2012; Gregorini et al., 2012).

Rumination has significant impacts on feed intake and forage utilization, which directly correlates to performance, health and welfare. Therefore it has been

proposed that rumination activity could be used as an indicator of animal health and welfare (Weary et al., 2009). Changes in rumination time could be used as a proxy measure of illness or changes in health status i.e. if detected, subtle changes in rumination activity could help in the detection of subclinical diseases before they progress and become a clinically apparent concern. To further investigate this possibility, accurate and precise methods to measure rumination time are required.

A detailed knowledge of feeding behaviour is also important in understanding the factors that can affect digestive function in ruminants. Very frequent measurements are necessary to record reliable information on feeding behaviour, but visual direct observations are expensive, laborious and require trained personnel. For the accurate study of feeding behaviour, long periods of activity must be recorded which can be made directly or by watching recorded videos (Kononoff et al., 2002; Martin et al., 1994; Mitlohner et al., 2001).

To overcome the difficulties of visual observation, various sensory devices have been used to record rumination by means of: detecting jaw movements, recording sounds of mastication or recording jaw movements and position of the head (Table 1.2). Automatic recording systems have the advantage of recording behaviours that can be easily missed by human observers, can be used in as many animals as there are devices available, and so in the long term the cost is relatively small. However these devices may be uncomfortable for the animal and could affect their normal behaviour. Also it is necessary for the equipment to be tested and validated to ensure that the obtained data is reliable and accurate.

In the past few years, the rumination collar (RC) (SCR Engineers, Israel) has been frequently utilised in the literature (Adin et al., 2009; Byskov et al., 2014; Gregorini et al., 2012; Hart et al., 2013; Schirmann et al., 2013; Soriani et al., 2012). The RC enables recording of rumination time from sounds recorded by a microphone with a neck collar, which is positioned to hold the RC's microphone on the left side of the cow's neck. The characteristic sounds of regurgitation and rumination are recorded, digitally stored, processed and then data presented as rumination time either min/2h or min/d (Bar and Solomon, 2010). The RC has been partially validated with both

dairy (Burfeind et al., 2011; Schirmann et al., 2009) and beef (Goldhawk et al., 2013) cattle.

The use of the RC was first assessed by Schirmann et al. (2009). This validation was carried out under controlled settings, by isolating the animals in individual pens to then be observed. The rumination time reported by RC was compared with that obtained by direct observations (Table 1.2). However the controlled conditions are not comparable to a commercial setting and further work was needed.

In a more recent paper by Elischer et al. (2013), a trial was carried out in a pasture based automatic milking system. The results of the RC performance were poorer than those reported by Schirmann et al. (2009) (Table 1.2). The authors proposed that this difference could be a result of the difficulty the human observers had when trying to record rumination i.e. cows were at a distance while at pasture and the position of the cows' head was not visible at all times. Furthermore the animal's free movement could have affected the RC position on the cow's neck, and the background noises of the grazing conditions could have interfere with the accuracy of the RC to record rumination.

Although the performance and output of the RC has been under scrutiny in the past years, the consensus seems to be that further validation is needed (Burfeind et al., 2011; Elischer et al., 2013; Goldhawk et al., 2013; Schirmann et al., 2009).

Table 1.2 Evaluation of different devices developed to measure chewing activity i.e. rumination and eating, in dairy and beef cattle.

Reference	Treatment	n	Animal	Variable	Device[1]	Observation[2]	r	R^2	P
Beauchemin	Pneumatic transducter[1]	5	Dairy	Rumination min/7h	75±6.1	76±5.1	0.83	NA	<0.01
et al. (1989)	Direct Observation[2]		cows	mean (±S.E.)					
Braun et al.	Halter pressure sensor[1]	10	Dairy	Rumination min/d	389.3±50.6	388.3±50.9	NA	NA	<0.05
(2013)	Direct observation[2]		cows	mean ±SD					
Burfeind et	Rumination collar[1]	15	Dairy	Rumination min/2h	24±14	28±16	0.88		<0.001
al. (2011)	Direct observation[2]		heifers	mean ±SD					
Elischer et	Rumination collar[1]	63	Dairy	Rumination min/2h	NA	NA	0.65	NA	<0.001
al. (2013)	Direct observation[2]		cows						
Goldhawk	Rumination collar[1]	119	Beef	Rumination min/2h	16.7±11.2	26.6±20.3	0.41		<0.01
et al. (2013)	Direct observation[2]		cattle	mean±SD					
Schirmann	Rumination collar[1]	15	Dairy	Rumination min/2h	35.1±3.2	34.7±20.3	0.96	0.93	<0.001
et al. (2009)	Direct observation[2]	36	cows	mean ±SD			0.92	0.86	<0.001

n= number of observations, [1]=measuring device, [2]= observation method. NA = no value reported.

1.3 Feeding strategies to improve or benefit rumen pH

With modern feeds and feeding practices, lactating dairy cows are subjected to rations which can result in a lower rumen pH. Several factors can affect the rumen environment, of which by far the most important is what the animal eats. Therefore several dietary strategies have been proposed to regulate rumen environment.

1.3.1. Dietary buffers

The acidic environment caused by modern feeding regimes can have a negative influence on animal performance. It is common practice in dairy nutrition therefore to resort to the addition of dietary buffers. The use of dietary buffers and alkalizing agents has often improved performance under such adverse conditions. Sodium bicarbonate ($NaHCO_3$), sodium phosphate, limestone, potassium carbonate (K_2CO_3) and bicarbonate ($KHCO_3$), and more recently the use of seaweed, are some buffers routinely used in the dairy industry (Cruywagen et al., 2015; Staples and Lough, 1989).

Erdman (1988) reviewed the use of several buffering ingredients such as $NaHCO_3$, magnesium oxide (MgO), $KHCO_3$ as fed additives. The author concluded that such agents are effective in increasing rumen pH, rumen acetate:propionate molar ratio and milk fat percentage in low forage diets and maize silage based diets. However the effects were less evident in diets containing more than 30% dry matter (DM) from forages (Erdman, 1988). The response of dairy cattle to the addition of dietary buffers in diets with high DM content reflects the capacity of the cow to assert control over rumen pH, by means of controlling intake and saliva production.

1.3.2 Inclusion of unprocessed grains and / or fibrous ingredients.

Ruminants require roughage in their diets to maximise production and to maintain health by sustaining a stable environment in the rumen. The cow chews feedstuffs to breakdown large particles, and the higher the fibre content the more mastication is

required, and therefore the higher the level of saliva is produced. Several studies have shown that increasing intake of fibre, especially neutral detergent fibre (NDF, i.e. cellulose, hemicellulose and lignin content), increases chewing activity and rumen pH (Beauchemin et al., 2003; Yang and Beauchemin, 2007; Yang and Beauchemin, 2006).

Differences in the amount and physical properties of fibre can affect the utilization of the diet and the performance of the animal. When too much fibre is included in the ration, energy density is low, intake and performance are depressed. However when too little fibre is included in the ration, rumen fermentation patterns are affected and hence altering digestion and animal performance. Furthermore rumen pH could decrease and reach deleterious levels (Mertens, 1997). Physically effective NDF (peNDF) is one indication of the potential or characteristic of a feedstuff to stimulate chewing and salivation. As defined by Mertens, (1997) the physical effectiveness factor (pef) of feeds ranges from 0 to 1.0; pef is multiplied by NDF content to determine peNDF content of the feed.

1.3.3 Multiple meals

Increased frequency events of food delivery and "push up" of available food will aid in the maintenance of a more stable rumen pH. More frequent bouts of shorter meals will prevent any sudden dips in rumen pH, and will maintain a stable rumen pH. Furthermore feeding PMR instead of separate ingredients could aid a more stable rumen environment by promoting longer meals. However sorting behaviour is still a resource employed by the animals that is hard to counteract. Animals alter their meal patterns and sorting behaviour in response in changes in management conditions e.g. time and frequency of feed delivery (DeVries and von Keyserlingk, 2008).

1.3.4 Enzymes

Fibre digestion in ruminants can only process a small portion of the available fibre, as between 20 to 70% of cellulose remains undigested (Varga and Kolver, 1997).

The application of exogenous fibrolytic enzymes to forages has been investigated as a method of enhancing fibre digestion and increasing milk production (Holtshausen et al., 2011). Fibrolytic enzymes are proposed to improve fibre digestion and alter chemical properties of the feed, hence having an effect on both feeding behaviour and chewing activity (Bowman et al., 2003). Most exogenous enzyme products are fibre-degrading enzymes that are products of microbial fermentation from bacterial (mostly *Bacillus* spp) or fungal (mainly *Trichoderma* or *Aspergillus* spp) origin.

1.3.5 Antibiotics

Growth promoting antimicrobials, such as ionophore antibiotics, have been widely utilised and are still used in some countries. Several studies have investigated the role of monensin (an ionophore) in preventing SARA (Mutsvangwa et al., 2002; Mutsvangwa et al., 2003). Contradicting results have been reported in the efficacy of monensin to aid in the manipulation of rumen pH by modifying microbial ecology and increase production of propionate (Fairfield et al., 2007; Plaizier et al., 2000). Virginiamycin is an antibiotic active against gram-positive bacteria (lactic acid producers), and the use of virginiamycin has reduced the risk of lactic acidosis, stabilizes rumen pH, and increases digestibility and energy utilization of grains (Clayton et al., 1999). However the use of antibiotics is a practice that might disappear due to concerns in public health, anti-microbial resistance, and food security (Pugh, 2002; Wegener, 2003).

1.3.6 Probiotics

The review by Fuller (1989) defines Probiotics as: “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance”. It is thought that probiotics offer some benefits and have been used as feed additives to enhance health and performance in livestock species (Chaucheyras-Durand and Durand, 2010). The inclusion of probiotics in animal nutrition is widely used in ruminants with the aim of optimising feed utilisation and rumen function,

improving DMI (Fuller, 1989; Krause and Oetzel, 2004) and animal performance (Robinson and Erasmus, 2009).

Probiotics used in dairy cattle nutrition are typically individual species or mixtures of lactic acid bacteria, yeasts or their end-products. These are presented as:

a) Live cultures of yeast or bacteria

b) Heat treated or inactivated cultures of yeast or bacteria,

c) Fermentation end-products from incubations of yeast or bacteria.

The most commonly used bacteria are: *Lactobacillus plantarum* and *Enterococcus faecium*, *Megasphera elsdenii*, *Selenomonas ruminanitum*, or their metabolic products have been use alone or in combinations with other probiotics (yeast) (Nocek et al., 2002b; Nocek and Kautz, 2006) to regulate rumen pH. Amongst the fungi species used are *Aspergillus oryzae*, *Aspergillus niger*. However the most common microbial fed to dairy cattle is the yeast *Sacharomyces cerevisiae* (Callaway et al., 2010; Chaucheyras-Durand and Durand, 2010).

1.3.6.1 Yeast supplementation

Although there are about 500 different species of yeast, the most common one used in cattle nutrition is *S. cerevisiae*. This can be presented as: live cultures of yeast; heat treated or otherwise inactivated cultures of yeast, and fermentation end-products from incubations of yeast. All of these probiotic categories have been used in various stages of lactation or growth in dairy cattle. Active dry (live) yeast is defined as containing no less than 15 billion live yeast cells colony forming units (cfu) per gram, being dried to preserve its fermenting power and containing no filler product. Its action depends upon the activity of the yeast in the rumen. Unlike live yeast, yeast culture products are defined as containing yeast and the media on which it was grown, and it can contain live cells or none at all. Yeast products rely on dead yeast cells, the media the yeast was grown on, and metabolites made by the yeast cell during the manufacturer's fermentation process to have a positive effect on rumen

fermentation. Fermentation by-products act through the supply of products of fermentation using yeasts (Callaway et al., 2010).

1.3.6.2 Response to yeast supplementation in ruminant diets

In recent years, several reviews on the supplementation of yeast on ruminant diets, especially dairy cows, have been published (Chaucheyras-Durand et al., 2008; Jouany, 2001; Robinson and Erasmus, 2009). On their review of the subject, Chaucheyras-Durand et al. (2008) elaborate on the use of live yeast supplementation modes of action, combination with other direct fed microbials, strain effect and selection. Desnoyers et al. (2009a) carried out a quantitative analysis of the literature by performing a Meta-Analysis of the effects of yeast supplementation on performance in different ruminant species. The authors collated data from more than 150 experiments and found that yeast supplementation had a positive effect on rumen pH, dry matter intake and milk yield. These effects on rumen pH were higher with diets containing higher inclusions of concentrate, and when higher DMI where observed. This positive effect was negatively correlated with the level of NDF in the diet.

In a similar Meta-Analysis, Poppy et al. (2012) assessed the effects of yeast fermentation products on performance of dairy cows. Mean differences between treatment (supplemented) and control groups were 1.18 kg / d and 1.61 kg / d for milk yield and 3.5% fat corrected milk (FCM) respectively. Milk fat yield and milk protein yield also showed an increase. Increments in DMI in early lactation studies were observed, and were lower in later lactation studies for treated cows (Poppy et al., 2012). The results of yeast supplementation have given conflicting results, ranging from a discrete improvement in performance to no significant effect. Production responses to yeast supplementation were variable, and ranged from 0 to 30% increase in milk yield across the entire lactation. However some authors report a better response within the first 100 days of lactation, possibly due to the “unstable” environment in the rumen during and after the transition period (Nocek et al., 2011). Bitencourt et al. (2011) reported a milk yield increase of 0.9 kg in yeast

supplemented cows compared to non-yeast supplementation, however there was no statistically significant difference. Erasmus et al. (2005) observed that mean milk yield, milk composition and body weight change did not differ between control and treatment (yeast supplemented) groups. Similarly the results reported by Dann et al. (2000) showed that milk produced during the first 140 days of lactation was not significantly affected by yeast supplementation.

Bruno et al. (2009a) found an increase of 1.2 kg per day in milk yield in cows fed a yeast culture compared with cows receiving a control diet with no yeast culture supplementation. However when compared for Energy Corrected Milk (ECM), no statistically significant differences were observed between control and treatment groups. It could be argued that the lack or minimal response to feeding a culture of *S. cerevisiae* on production of ECM was due to a reduction in the concentration of milk fat observed when cows were fed yeast culture compared with controls (Bruno et al., 2009a).

It could be that any potential beneficial effects of yeast supplementation are lactation stage, diet and environmental condition dependant. Robinson and Erasmus (2009) found a consistent response in milk yield to yeast supplementation in the meta-analysis they carried out. However they also found several factors that can limit the effect of yeast supplementation, these being: 1) the higher the milk yield, the lower the response to yeast supplementation, 2) increasing levels of NDF in the diet had a strong negative impact on the response of the cows supplemented with yeast in terms of milk yield, milk protein and DMI. Additionally Yang and Beauchemin (2005) reported that increasing the ADF of the diet had an even stronger effect than NDF on suppressing response to feeding yeast.

Response to yeast supplementation on rumen fermentation parameters are diet-dependant. Opsi et al. (2012) found the response to yeast supplementation is more noticeable when associated with high-fibre substrates, with more subtle effects observed on high-concentrate diets. Yeast tended to increase rumen pH (Krizova et al., 2011; Richter et al., 2010) in dairy cows. However no effect of yeast

supplementation was observed on rumen pH in other experiments in dairy goats (Desnoyers et al., 2009b) or in cows (Julien et al., 2010).

The metabolic activities of the yeast strain and survivability throughout the gut appear to be of great importance for an optimal efficacy (Chaucheyras Durand 2008 and Newbold 1995).

It is thought that yeast affects rumen ecology, creates competition with bacteria for substrates, and stimulates the growth of other types of rumen microbes such as lactate utilizing bacteria, as well as stimulating the growth and activity of cellulolytic bacteria. By consuming oxygen from the rumen environment (Newbold et al., 1996), yeast promotes an anaerobic environment and provide vitamins, amino acids and other nutrients hence improving the rumen milieu for fibrolytic bacteria and other microorganisms to multiply (Chaucheyras et al., 1995; Marden et al., 2008). It is also argued that yeast attaches to feed particles and interacts with other microorganisms forming what is called a “microbial consortium”. This improves the uptake of nutrients and increases cell wall digestion by helping with bacterial attachment and colonization of feed particles (Figure 1.4) (Jouany, 2006; Jouany, 2001).

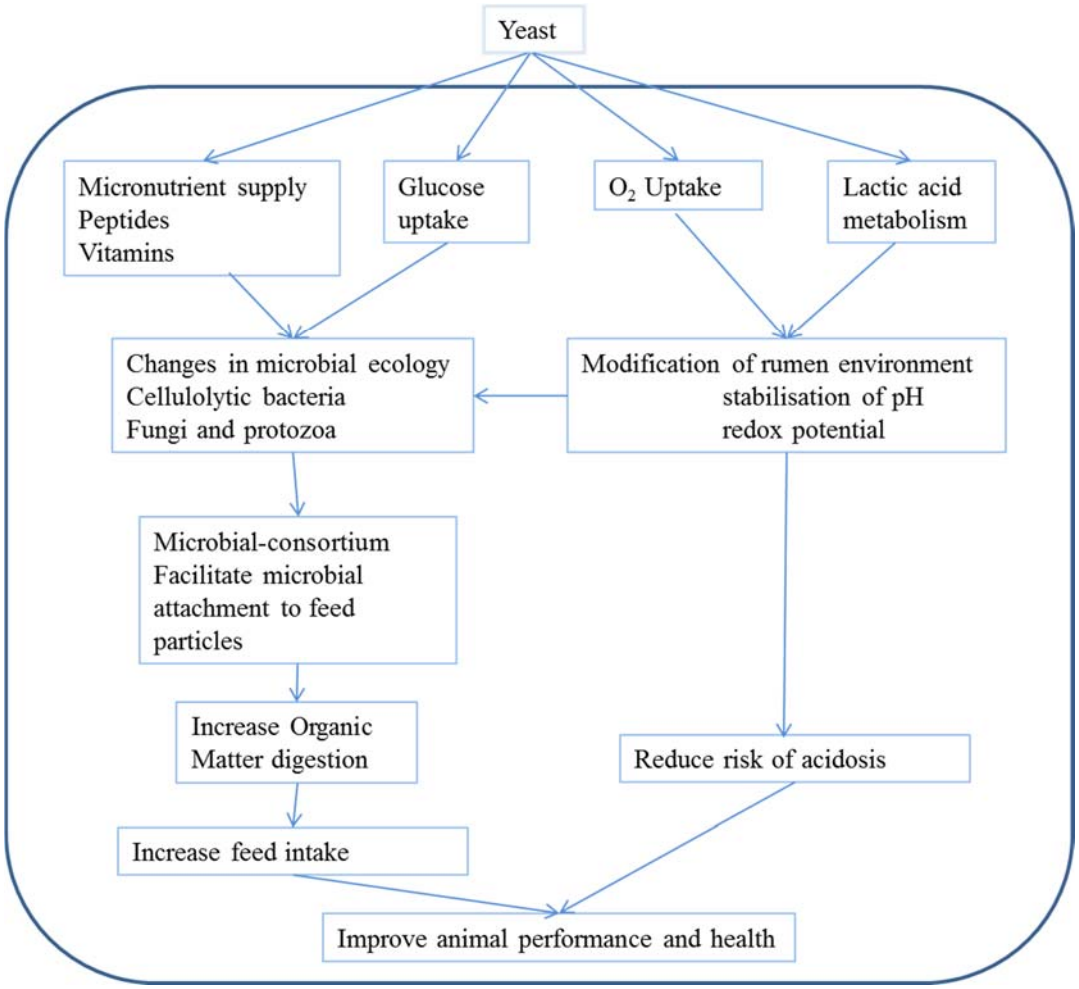

Figure 1.4 Theoretical main effects and mode of action of yeast, *Sacharomyces cerevisiae* (modified from Jouany, (2006))

High concentrate diets tend to result in the accumulation of lactate that is generally associated with rumen acidosis. Yeast affects the quantity of lactic acid (Julien et al., 2010; Marden et al., 2008) which can aid in preventing the reduction of rumen pH and increasing nutrient digestibility. Feeding yeast products may be most beneficial to dairy cows during late gestation and early lactation when cows experience the highest levels of physiological challenges. The transition period around calving is a crucial period due to increases in milk yield in relation to nutrient intake, changes in nutrient density and fibre content within the diet, and reduced DM intake which challenges nutritional status.

Overall it is thought that yeast promotes a more stable rumen pH. Cellulose digestion in the rumen is depressed at a pH level below 6. Higher and more stable rumen pH allows fibre digesting microorganisms to remain fully active, increasing efficiency of digestion and hence productivity. This will result in a higher supply of energy to the cow, which in turn will increase milk yield and milk components. Feeding yeast might alter rumen fermentation and increase fibre and DM digestibility, with subsequent increased supply of absorbed nutrients for milk synthesis (Bruno et al., 2009a).

In vitro studies indicate that the incorporation of a culture of *S. cerevisiae* altered microbial fermentation and increased digestibility of DM and crude protein (CP) (Opsi et al., 2012), which has been suggested to favour microbial growth in some but not all *in vivo* studies. Furthermore *in vitro* studies in which *S. cerevisiae* was supplemented to the culture media suggested changes in the rumen microbial population that might favour a more stable rumen environment. Therefore improvements in animal performance because of the addition of S. *cerevisiae* cultures are likely to result in an increased rumen digestibility of DM and the fibrous fraction of the diet, changes in the supply of metabolisable protein, and improved stability of rumen pH, which might favour small increases in DM intake and supply of energy for milk synthesis.

1.4 Mathematical modelling in dairy nutrition

During the past several years, milk yield per cow has increased, which in turn signifies an increment in dry matter intake (DMI) to account for the increasing nutrient requirement to achieve such high milk yields. However, the rate of increase in energy requirements has increased more rapidly than DMI, thus leading to diets with higher nutrient density (Eastridge, 2006). The increase in energy density of diets place challenges for dairy cow nutrition, not only to be able to develop suitable diets targeting high yields, but also because feeding energy dense rations can increase the susceptibility of dairy cows to suffer from SARA. Furthermore the continuously increasing cost of feedstuffs and low prices paid for milk provide a strong impetus in

dairy nutrition to improve feed efficiency (feed conversion to milk), which will have significant impacts on the profitability of dairy production.

Models relating dietary inputs and animal performance are required to devise feeding strategies that minimise feed cost, while maximising animal performance and reducing environmental impact. The capacity to integrate all the required knowledge into a model can be used to assist decision making (Black, 2014). Mathematical models have been used to predict DMI (Roseler et al., 1997; Vadiveloo and Holmes, 1979), growth and performance (Baldwin et al., 1987a; 1987b; 1987c; Fox et al., 1999; Friggens et al., 1999) and are now integral to animal science and dairy nutrition. These models have become the basis of the animal requirements systems (AFRC, 1993; NRC, 2001) and feeding or ration formulation programmes (Fox et al., 1992; O'Connor et al., 1993; Russell et al., 1992; Sniffen et al., 1992; Thomas C, 2004). By calculating nutrient requirements to account for a determined milk yield target (and other physiological functions) these models enable its users to formulate rations by adding different feed ingredients hence predicting energy and protein available to achieve performance targets.

Despite the impact of low rumen pH on farm profitability and animal welfare, and although these models and ration formulation programmes might be capable of predicting it as part of their calculations, they do not have rumen pH as one of their main outputs (Mills et al., 2014). Several models have been developed that try to predict rumen pH (Fox et al., 2004). These models are mostly empirical equations, stand-alone models/equations or part of "bigger" more complex models i.e. rumen model or whole animal models (Table 1.3). These models can be described as empirical or mechanistical models.

Table 1.3 Models to predict rumen pH

Reference	Animal	Model	Equation	R^2
Allen (1997)	Dairy	Rumen / Animal	pH = 6.56 – 0.0049xVFA	0.13
Argyle and Baldwin, (1988)	Dairy	Rumen / Animal	pH = 7.2 – 0.01 cVFA + 0.0015 x cLa	NA
Fox et al. (2004)	Dairy / Beef	Rumen / Animal	pH = 5.425 + 0.04229(peNDF)[1]	
Lescoat and Sauvant, (1995)	Beef	Rumen	pH = 7.56 – 0.0131(VFA)	0.80
Mertens (1997)	Dairy		pH = 6.67 – 0.143(peNDF)	0.71
Mills et al. (2014)	Dairy	Rumen	$pH = 7.73 – 0.014C_{VFA} – 0.0154C_{LA}$	NA
Pitt et al. (1996)	Dairy, beef and sheep	Rumen / Animal	pH = 5.46 + 0.038(eNDF)[2]	0.52
Tamminga and Vanvuuren (1988)	Ruminants		pH = 7.73-0.014VFA	0.71
Zebeli et al. (2008)	Dairy		pH = 5.59 + 0.0218(peNDF)	0.50

VFA = Volatile fatty acids, VFA (mmol), CVFA = concentration VFA (mmol/l), CLA = concentration lactic acid (mmol/l), [1]= equation used when peNDF<24.5% or else pH = 6.46, [2] = equation used when eNDF < 26.3% when eNDF >26.3% pH = 6.46, NA = Not available

1.4.1 Empirical models.

These models aim principally to describe the responses of a system, often using mathematical or statistical equations. These models are constructed by regression analysis of measured variables that might have an effect on the component under study. It is argued that empirical models are constructed without any scientific content and unconstrained by any scientific principles i.e. they imply little about the system, and do not try to explain the underlying mechanisms controlling the system (Black, 2014; Thornley and France, 2007). Predictions from this type of model are frequently poor when applied to data outside their parameterisation range. However these models are a means to test basic components and use variables that are most of the time easily recorded at a practical on-farm level or as a result of other equation models.

Several models have been constructed trying to predict rumen pH, predominantly by exploring the relationship (using regression equations) of different variables with pH; feed characteristics (Mertens, 1997; Pitt et al., 1996) and VFA concentrations in rumen fluid (Allen, 1997; Tamminga and Vanvuuren, 1988) being the main means of prediction of rumen pH. In a recent review of different models that predict rumen pH in beef animals, Sarhan and Beauchemin (2015) evaluated eight models (Table 1.3) for their accuracy to predict rumen pH. The authors found in general poor results when comparing predicted versus measured rumen pH values for all the evaluated models (R^2 = 0.10 – 0.52, concordance correlation coefficient CCC = 0.22 – 0.67). The model reported by Pitt et al. (1996) was the model that performed best in this exercise (R^2 = 0.10 – 0.52 CCC = 0.67). With the results obtained, the authors concluded that the ability of the models to predict rumen pH was low, and further work was required to explore and incorporate other factors that might affect rumen pH (other than VFA and peNDF) to help increase the accuracy of model predictions. Empirical models alone do not fully encompasses the complexity of factors affecting rumen pH.

1.4.2 Mechanistic models

Mechanistic models provide a degree of understanding or explanation of the phenomena being modelled. The term "*understanding*" implies a causal relationship between the quantities and mechanisms (processes) which are represented on the lower level, and the phenomena which are predicted at the upper level. For example, milk yield can be interpreted in terms of the operation of the process of energy (ketogenic or glucogenic) metabolism (lower process). A mechanistic model is based on ideas of how the systems works, what the important elements are and how they relate or interact to each other (Thornley and France, 2007). Mechanistic models have been used to describe a range of animal systems, including nutrient metabolism and energy transactions in individual organs or whole animals.

Several reviews on mechanistic models in dairy science have been carried out in the past dealing with mechanistic models of organs (Offner and Sauvant, 2004) or whole animal models (McNamara, 2004; Tedeschi et al., 2005). Offner and Sauvant (2004) evaluated three different mechanistic models representing the rumen MOLLY (Baldwin et al., 1987c), the rumen model within the Cornell Net Carbohydrate and Protein System (Fox et al., 1992; Russell et al., 1992; Sniffen et al., 1992) and the model developed by Lescoat and Sauvant (1995). The model performed with acceptable results for different predicted variables (starch and fibre digestion in the rumen, microbial dynamics, VFA production and rumen pH), and the authors concluded that the models could be improved by taking the advantages from each model to improve the others.

McNamara (2004) deconstructed and explained the relevance of mechanistic models by means of describing nutrient utilization in dairy cattle. The ability to describe metabolic function, pathways, and their resultant effect on nutrient requirements is critical to the continued ability to raise food producing animals in efficient ways around the world. The author emphasises that empirical systems have been adequate to date. However as progress in science and research is achieved, it is only through continuing to develop models of increasing complexity that true knowledge will arise (McNamara, 2004).

Research by Baldwin (1987a; 1987b; 1987c) enabled the development of mechanistic mathematical models of animal metabolism, which aim on the one hand to improve our understanding and integration of knowledge for research reasons, and on the other hand to improve predictions for practical purposes. Their broader biological aims and reliance on more than one data set for parameterization allows their wider application than traditional empirical models. However they have not been widely adopted in commercial nutritional formulation programmes (Hackmann and Spain, 2010).

Mechanistic mathematical models have been developed that take into account the site of feed digestion, the type of nutrient absorbed and the type of nutrients required for production, thus providing a better prediction of the effect of different feeding strategies on rumen pH dynamics (Dijkstra et al., 2008). Black (2014) describes the general structure of these models: a description of the animal is required in terms of its genetic potential and physiological state, and a description of the diets available for consumption. Then intake is predicted using different types of equations, from which digested feed and available nutrients for metabolism are predicted, and partitioning of those nutrients to different body functions is allocated. From all these calculations, predictions of different outputs are obtained as accumulation of protein, fat, milk yield, excretion of nitrogen methane and other variables.

As mentioned previously, despite the impact of low rumen pH in dairy cows, mechanistic models and ration formulation programmes do not have rumen pH as one of their main outputs (Mills et al., 2014). Rather rumen pH is used to affect other outputs of the model. Rumen pH can reduce rumen fibre degradation thus affecting its nutritive value. A decline in fibre degradation will reduce the amount of VFA produced in the rumen and microbial synthesis, hence the predicted supply of nutrients (Dijkstra et al., 2012). Nevertheless mechanistic approaches of dairy cow nutrition evaluation might address the relationships between host animal, feed and rumen microorganisms, and demonstrate a greater capacity for describing ruminal fermentation processes that determine rumen pH.

1.5 Aims of the thesis

Due to current feeding regimes used in the dairy industry, there is major concern for the occurrence of SARA with its associated deleterious effects on dairy herd health and performance. Furthermore with the development of new technologies, the necessity for novel accurate methods that aid in monitoring, diagnosis and potential treatment options in modern large dairy herds becomes available.

The use of direct feed microbials is a common practice in the dairy industry, amongst others for the treatment and prevention of SARA. However given the conflicting research results obtained with some of these products (e.g. yeast supplementation) on dairy cow performance and rumen environment as a strategy to counteract SARA, more investigation is warranted. This thesis therefore sought to improve monitoring of rumen health in dairy cattle, and investigate the efficacy of yeast supplementation to reduce the risk of SARA under standard commercial farm conditions.

The aim was to perform experimental work in commercial on-farm environments under standard conditions, to allow the direct translation of this work into practice.

The objectives of the thesis were therefore to:

1.5.1 Assess various methods to monitor rumen health in dairy cattle using novel technologies such as rumination collars and rumen pH boluses

1.5.2 Investigate the effect of yeast supplementation on performance, rumination activity and rumen function in lactating dairy cows

1.5.3 Evaluate empirical modelling and a commercially available whole cow mechanistic model in dairy cow nutrition for the prediction of rumen pH

Chapter 2 Material and Methods

2.1 Trials structure

All the on-farm trials were conducted at the University of Edinburgh at Langhill Dairy Farm, Roslin (Midlothian, Scotland UK) during 2012 and 2013. The farm has a 240-cow Holstein milking herd. All procedures related to animals were approved by the Veterinary Ethical Review Committee (References: Trial 1 VERC 2011-88, Trial 2 VERC 30/12, and Trial 3 VERC11/13) of the Royal (Dick) School of Veterinary Studies of the University of Edinburgh.

In each trial fourteen multiparous milking cows were selected and balanced for days in milk (DIM) and parity (3rd or more lactations). Individual cows were unique to each trial, once selected the cows were then randomly allocated to two different groups: group 1 (G1) and group 2 (G2), with seven cows in each group. Cows were divided into 2 groups to facilitate management routines (e.g. milking and video recording), and to ensure similar parities and DIM between groups of cows. Each group was housed in contiguous pens that shared identical characteristics: area of feed and water troughs, cubicle/stalls with rubber mattresses top-dressed with sawdust 3 times a week. In Trial 3 cows were grazing grass.

Cows were milked in a 14 / 14 herringbone milking parlour (DeLaval, Cardiff UK) at approximately 0500 and 1500 h. During milking cows received a minimum of 0.8 kg and a maximum of 6 kg of concentrate a day per cow. Concentrate usage was 0.4 kg / litre of milk.

Milk yields were recorded daily, and downloaded once a week directly from the milking parlour using the automatic milk recording feature: the Alpro 5 computer programme (DeLaval, Cardiff Wales UK). Daily milk yields were determined using the "yesterday yield" feature.

Composite milk samples per cow were taken at each milking (a.m. and p.m. milking) during the measurement weeks of each trial. From this recording, levels of butterfat,

protein and somatic cell counts per cow were determined by Cattle Information Service (Cattle Information Service (CIS), Herts, England UK). Data was downloaded onto a computer from DataStream disc, onto UNIFORM-Agri (Somerset, England UK) software programme.

Cow weight was recorded using an electronic weigh scale.

Cows were body condition scored (BCS) by one trained operator to ensure consistency on the recording, according to the standard 1 – 5 BCS scale, in which 1 is emaciated and 5 is obese (Lowman et al., 1976).

Fertility and cow health events / issues were recorded and kept on UNIFORM-Agri (Somerset, England UK) software programme.

Cows were offered a partial mixed ration (PMR) consisting mainly of wholecrop wheat and grass silage. Rations were fed once a day (around 0800 h) using a Keenan Klassik II 115 EF feeder wagon. Ration was fed so that 5 – 10 % was refused each day, which was then removed from the trough before fresh ration was put down the following day. Additional concentrate was fed dependant on milk yield in the milking parlour. Water was supplied ad libitum. In Trial 3 cows were grazing grass and a PMR buffer diet was offered and available to the cows after the afternoon milking (from 1500h approximately for two hours).

Forage samples were taken every week and were analysed at Bioparametrics Ltd. (Edinburgh, Scotland UK) laboratory. Basic components of the feedstuffs: dry matter (DM), Ash, Oil, Sugar, Starch, NDF, Protein and fermentation products (volatile fatty acids (VFA)), lactic and ammonia) were obtained by AOAC International methods (AOAC, 2012). Further analyses which are unique to Bioparametrics Ltd. feedstuff analyses included: degradation parameters of the carbohydrates and protein performed by *in vitro* gas production technique (IVGPT) (Menke and Steingass H., 1988) with modifications for protein and carbohydrates (Jessop and Herrero, 1996; Palmer, 2006). The results of these analyses provide information on the degradation parameters of the carbohydrates, sugar, quickly and slowly degradable starch, fermentable NDF and protein fractions of the feedstuffs.

2.2 Behavioural measurements

All individual cows were clearly identified with a unique number or letter by colour spray (Arco Limited, Hull UK) on either side of the thorax, neck, or both so they were easily viewed and recognized (Figure 2.1a). Behavioural measurements were recorded using video cameras (Figures 2.1b and 2.1c) or with direct visual observations.

2.1a 2.1b 2.1c

Figure 2.1a,b,c. Images of the cows on the shed. The red circles show the video cameras fitted to the roof and the blue circles show the unique numbers used to identify each individual cow (a). Equipment used to analyse the video recordings (b) and a close-up of the display that shows the unique number on the cow easily identifiable and the date and time on which the recording was made (c).

2.2.1 Video recordings. Cow behaviour was recorded using 16 video cameras (Panasonic WV-LF4R-5C3AE, Panasonic Bracknell England UK, Figure 2.1a) with 1/3" fixed iris lenses (Panasonic WV-LF4R-5C3AE, Panasonic, Bracknell England UK). The cameras were positioned in key places throughout the shed (fitted to the roof 4.0 and 5.5 m above the ground) so that all cows were viewed and easily identified (by their unique number or letter) at any given time. The area under observation was naturally lit during daylight hours and infrared lighting was used for night time recording. The cameras recorded 24 h / d. On an average day, 3 h of cow behaviour was missed as the cow left the pens to be milked (around 0500 and 1500 h). The signal from the cameras went through a video multiplexer (Panasonic WJ-

FS616) to record 24hrs onto a single 4h VHS video tape using a time-lapse video cassette recorder (Sanyo SRT-8960P). New video tapes were changed every day at the same time: only about 30sec of behaviour was lost due to the exchange from a used video to the new one.

Behavioural measurements were analysed and recorded using The Observer software (Noldus Information Technology, 2004, Wageningen, the Netherlands) by one trained observer using the recorded videotapes.

2.2.2 Direct observations. Cow behaviour was recorded by one trained observer using a handheld device (Psion WorkAbout Pro M, Noldus Information Technology). Cow behaviour was recorded continuously without interfering with their normal behaviour:

a) when cows were housed indoors, the observer was standing in places of the shed where all the behaviours of a specific animal were easily recorded, and the observer's presence had no effect on the cow's routine and behaviours (i.e., the animal did not change behaviour or move away from the observer);
b) when cows were outside grazing on pasture, the observer was standing in the field at a distance (approximately 10 m) where all the behaviours of a specific animal were easily recorded, and the observer's presence had no effect on the cow's routine and behaviours (i.e., the animal did not change behaviour or move away from observer).

Behaviours (eating, drinking, idling and ruminating) were recorded according to the ethogram shown in Table 2.1. Using focal animal sampling behaviours were recorded continuously (Martin et al., 1994; Mitlohner et al., 2001) and were defined as being mutually exclusive categories.

Table 2.1: Behavioural ethogram used in Trials 1-3.

Behaviour	Definition
Eating	Head over or in the feed trough
Drinking	Head over or in the water trough
Ruminating*	Time the cow spends chewing a regurgitated bolus until it swallows it back
Idling	No ruminating, eating or drinking behaviour

*Ruminating was recorded with the cow standing or laying as the same behaviour.

2.3 Rumination Collars

At the beginning of each Trial, a rumination collar ((RC) Qwes-HR Lely Ltd., St Neots UK) was fitted to each cow to record rumination (Figure 2.2). The RC enables the recording of rumination time from sounds recorded by a microphone with a neck collar, which is positioned to hold the RC microphone on the left side of the cow's neck. The characteristic sounds of regurgitation and rumination are recorded, digitally stored, processed, and then data presented as rumination time either in min / 2 h or min / d (Bar and Solomon, 2010).

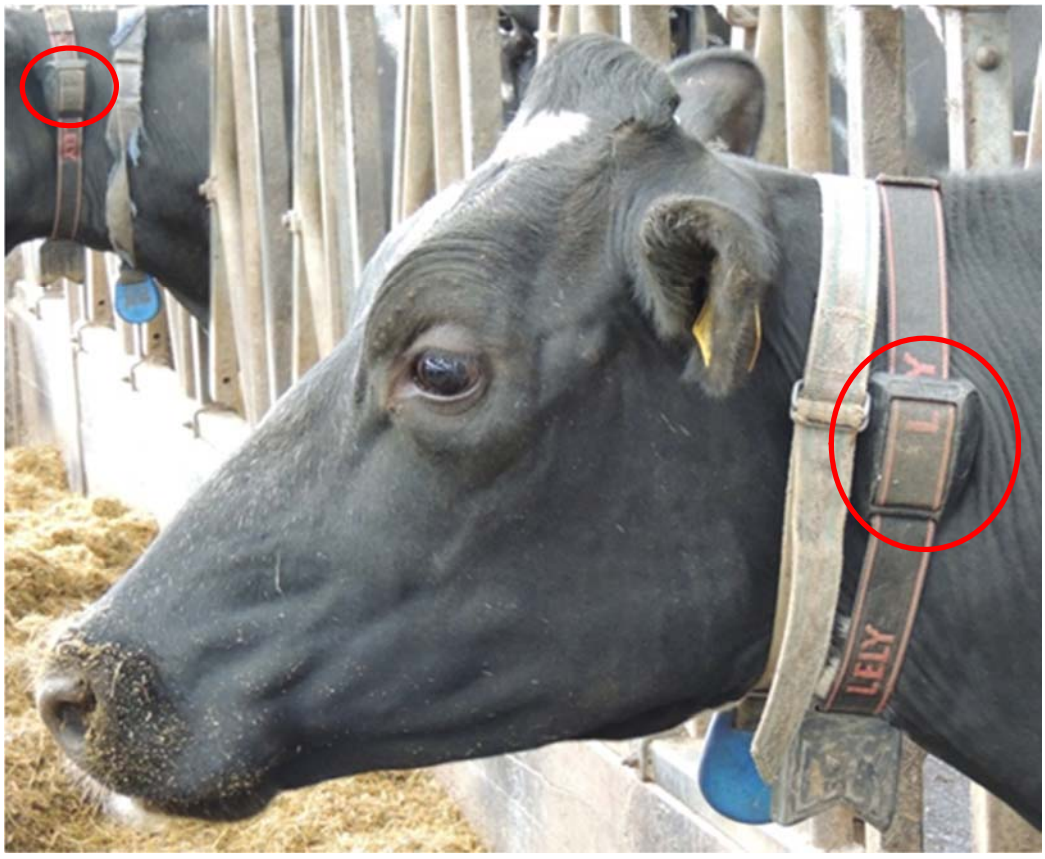

Figure 2.2. A rumination collar was fitted to each cow to record rumination. The red circles show the tag that contain the microphone that enables the RC to record the characteristics sounds of mastication.

A tag reader was located at the exit of the milking parlour so data from the RC were downloaded to and stored at least twice a day, after each milking. This prevented overwriting of the data because the RC internal memory capacity has only a 21-h storage capacity. The raw data from the RC were then collated. The output presents rumination in minutes per 2-h periods (e.g. 0200 h, 0400 h, 0600 h or 0100 h, 0300 h, 0500 h, and so on) over a day. The RC was fitted to each cow at the begging of each Trial and data was recorded immediately after the RC was fitted. Data used for analyses was that from day one of the measurement week.

2.4 Rumen boluses

To record rumen pH, cows were orally administered an intra-ruminal bolus (eCow Limited, Devon, England UK or WellCow Limited, Roslin, Scotland UK) Trial 1 = eCow (Figure 2.3a) and Trials 2 and 3 = WellCow (Figure 2.3b).

The bolus consists of a sensor, electronics component to transduce and condition the signal and store the data, a radio transceiver, aerial and battery all sealed within an enclosed container (Mottram et al., 2008). The pH and temperature sensor is encapsulated on a cylindrical bolus of approximately 32 x 145 mm size and 240 g of weight.

Prior to deployment the boluses were calibrated against known standard pH solutions (pH 4 and pH 7, Trial 1 = Osmotics, Aylsham, England UK and Trials 2 and 3 = buffer solutions were provided by WellCow Ltd.). Rumen boluses were set to record pH at 15 min intervals for the entire lifespan of the bolus's battery (up to 4 months). The devices have the capability to store the information for up to one month. However data was downloaded every week to prevent losing the recorded data. The boluses were set on recording mode before deployment. Manufacturers of the boluses utilised state that the boluses reside in the reticulum (Mottram et al., 2014). The rumen boluses were deployed and administered to each cow one day before the staring of the first period's measurement week at each of the Trials. Data used for analyses was that from day one of the measurement week.

2.3a 2.3b

Figure 2.3 Rumen bolus: eCow (a) and WellCow (b).

2.5 Dietary interventions

In all three trials yeast was supplemented to evaluate its effects on rumen pH, rumination behaviour and performance. Cows were supplemented with yeast at two different rates (0.8 and 4.0 g/cow/d, Vista Cell, AB Vista). The yeast was fed top dressed to the PMR in 50g of wheat, which was then mixed with the PMR just after morning feeding. Cows on the Control group (no yeast supplementation) received 50g of wheat (placebo) only.

2.6 Statistical Analysis

Data was collated and summarised to the relevant unit for further analysis *e.g.* mean pH per day or mean pH per hour for rumen pH, daily milk yield, mean milk characteristics per period etc.

Data on body condition score (BCS) was analysed using Kruskal-Wallis one way ANOVA.

Somatic cell count was transformed using Log_{10} and was analysed using a mixed effect model.

To evaluate reliability when assessing two variables by two different methods Pearson correlation coefficient, regression analysis and standard Limits of Agreement (LoA) method were calculated.

When evaluating the relationship of two variables measured with repeated measurements, a modified version of the LoA methodology, and a linear mixed effect model were performed. The results of this analysis are presented as least squares means (lsmeans).

All statistical analyses were carried out using R (R Core Team, 2013). Statistical significance was taken as $P < 0.05$. Further details of each analysis and specific statistical packages utilised are given in the relevant section of each Chapter.

Chapter 3 Results

On-farm trials: Effect of yeast supplementation under various conditions in commercial dairy cows

3.1 Introduction

The ruminant nutrition industry is continually searching for alternatives to enhance production, or to correct potential issues that may reduce productivity. Direct fed microbials for example bacteria and yeast, have been used as additives to improve rumen function and performance. *Saccharomyces cerevisiae* is the most common yeast currently supplemented to dairy cows. The use of yeast cultures (Dann et al., 2000), inactivated yeast (Fortina et al., 2011) or various different strains of live yeast (Moallem et al., 2009; Nocek et al., 2003) is common practice as these products are commercially available. Several studies have looked at the effect of yeast supplementation (as live yeast or yeast products) in dairy cows on rumen environment (Hristov et al., 2010; Kung et al., 1997), performance (Bruno et al., 2009a; Kalmus et al., 2009; Kung et al., 1997) and health (Bruno et al., 2009b). However despite several decades of research into the use of yeast as a feed additive for ruminants, the results have been inconclusive and the proposed benefits of yeast supplementation have not always been demonstrated. For example some studies reported no effect of yeast supplementation (Schingoethe et al., 2004), or a trend on increased performance (Dann et al., 2000) or significant effects of yeast supplementation (Desnoyers et al., 2009a). It is thought that the variable response to yeast supplementation is a result of external factors such as diet, physiological stage, or type of yeast fed (live or yeast product).

Studies have focused on the effect of yeast supplementation under experimental conditions (induction of SARA), or adverse environments (environmental heat stress or physiological early lactation). However it is very seldom that studies have looked at the effect of yeast supplementation under standard commercial conditions.

Therefore the aim of the present study was to evaluate the effects that yeast supplementation may have on cow performance, rumination time and rumen pH in cubicle housed and grazing commercial dairy cows.

The Trials presented here are novel in that to our knowledge no other studies have looked at the effect of yeast supplementation on circadian rumen pH and rumination activity as a measure of rumen health on commercial dairy cow.

3.2 On-farm trials

Three trials were conducted at the University of Edinburgh at Langhill Dairy Farm, Roslin (Midlothian, Scotland, UK) during 2012 and 2013. All procedures related to animals were approved by the Veterinary Ethical Review Committee of the Royal (Dick) School of Veterinary Studies at the University of Edinburgh.

Langhill dairy farm has a 240-cow Holstein milking herd, and the farm management is similar to that observed on commercial UK dairy farms. An overall description of the three Trials will be given, with further details on specific differences to each individual Trial provided later in its respective section.

3.3 Materials and methods

3.3.1 Animals and Housing

Fourteen multiparous milking cows were selected and balanced for DIM (mean ± SEM) and parity [median lactation number (L)]. The cows were then randomly allocated to two different Groups of seven cows each to facilitate management routines (e.g. milking, feeding and yeast dosing). It also ensured similar parities and DIM between groups of cows in all 3 Trials. Each group was housed in contiguous pens that share identical characteristics: area of feed and water troughs, cubicle/stalls with rubber mattresses top-dressed with sawdust 3 times a week. All individual cows were clearly identified with a unique number or letter by colour spray (Arco Ltd.,

Hull, UK) on either side of the thorax, neck, or both so they were easily viewed and recognized.

3.3.2 Experimental design

The Trials were split in Periods of 3 to 4 weeks duration. The animals were given a minimum of two weeks to adapt to the facilities and diets, and all measurements were recorded in the last week of each Period. An example of a 3 week experimental Period is presented in Table 3.1.

3.3.3 Diets and yeast supplementation

Cows were fed once a day at approximately 07:00 am when fresh partial mixed ration (PMR) was delivered. Additional concentrate was fed to yield in the milking parlour, and cows received a minimum of 0.8 kg and a maximum of 6 kg of concentrate a day per cow according to milk yield. Water was supplied *ad libitum*. When required by the experimental design, cows were fed live yeast product (12.5 billion cfu /g, VistaCell, AB Vista, Woodstock Court, Marlborough Business Park, Marlborough UK). The yeast is a commercial product routinely fed as part of dairy cows diets in the UK. The yeast was top-dressed or mixed on the PMR after fresh food was delivered. Cows were locked in the feed trough for 15 min using the self-locking neck yolk mechanism to ensure that the cows ate all of their yeast allocation.

Table 3.1 Experimental period outline and measurements performed.

Groups	Period		
	Week 1 Adaptation	Week 2 adaptation	Week 3 measurements / recording
Group 1 and 2			Milk yield daily Rumination daily (min / 2 h or min / 24 h) Rumen pH every 15 min Milk composition Monday, Wednesday and Friday BCS and BW once a week Feed sampling once a week

3.3.4 Measurements and Sampling procedures

Table 3.1 summarises all the measurements recorded during the measurement week:

Milk production and milk composition

Cows were milked as described previously in Chapter 2. During the measurement week of each Period (on Monday, Wednesday and Friday), individual milk samples were collected from consecutive morning and afternoon milkings to be analysed for chemical characteristics (Table 3.1).

Body weight and body condition score

Once during the measurement week of each Period, cow weight and BCS were recorded as described in Chapter 2.

Milk yield, cow body weight and BCS were unknown before the starting of each Trial.

Rumen pH data collection

To record rumen pH, cows were orally administered a commercially available rumen bolus. A bolus was administered to each cow using a bolus gun before the start of the first measurement week on the first period. To avoid losing data, the recorded rumen pH data was retrieved from the boluses every week and stored on a personal computer for further analysis.

Rumination time data collection

At the start of each Trial and for the whole duration of the Trial, a RC was fitted to each experimental cow to record rumination time. The RC have been described elsewhere (Bar and Solomon, 2010) and have been validated for its use in loose housed dairy cows in commercial environments with acceptable results (Ambriz-Vilchis V. et al., 2015).

Feed sampling and analysis

Exact details of the PMR and the parlour concentrate composition were obtained. Samples (500 g approximately) of the forages used in the PMR were taken every measurement week and were analysed for basic chemical characteristics and fermentation parameters at Bioparametrics Ltd. laboratory (Edinburgh, Scotland UK).

3.3.5 Data collection and Statistical Analysis

Milk yield and composition

Data on milk yield and characteristics, BCS and BW was collated and summarised for the measurement week resulting in a dataset for analyses of: seven measurements for milk yield (one per day), three measurements for milk characteristics (Monday, Wednesday and Friday) and one measurement for BCS and BW. Malfunctions on the milk meter recording device at the milking parlour were occasionally reported, and therefore milk yield data was screened for inconsistencies i.e. low or missing values. Any daily milk yield measurement that was less than 20% of the value on the previous and following measurement day was highlighted for investigation, and if necessary was removed from dataset. When possible, it was replaced by an average value taken from the previous and following date. Data on sick cows (e.g. cows diagnosed with mastitis) was taken into account by cross-checking with veterinary and farm treatment records, and their data was removed from the dataset.

Rumen pH data collection

Rumen boluses were set to record pH every 15min, which resulted in 96 time points per day. Daily pH records were examined on an individual cow basis, and only days that had 96 time points were utilised to construct the pH database. Values were consider outliers and were omitted from the dataset when consistently unusual values

were recorded i.e. drift on the values higher than 7.0 or lower than 5.0 as this patterns represented a faulty pH sensor.

From the dataset obtained, rumen pH data was presented as:

a) Mean pH per hour. An average value was obtained from the 4 measurements obtained per hour, resulting in 24 mean pH data points per day.
b) Time (min / d or h / d) spent below 6.2 or 5.8 pH thresholds. These cut-off points were selected as indicators of Sub Acute Rumen Acidosis (SARA). These rumen pH values were considered due to research evidence showing their detrimental effects on ruminal cellulolytic bacteria and fibre digestibility (pH < 6.2) (Mould and Orskov, 1983; Russell and Wilson, 1996) and / or direct harmful action on the animal host (pH < 5.8) (Beauchemin and Penner, 2009).

Rumination time

Data obtained with the RC was collated to present rumination time in minutes per 2-h periods for each day of the recording week. Occasionally malfunctions with the communication between the RC and the stationary reader occurred and data was overwritten and lost. Therefore only days of data that reported the entirety of a 24 h day was used to build the rumination time database. Rumination time values on the database were further summarised as total rumination (min per day) for each day during the measurement week.

Statistical Analysis

To analyse milk yield, milk characteristics (BF, MP, LAC, FCM), rumen pH and rumination time data, a standard linear mixed-effect model was used to resolve the non-independence associated with the multiple measurements recorded per cow. In the linear mixed effect model, which cow that the measurement had come from was entered as the random effect. The model included fixed effects of period and treatment, and the interaction of period and treatment with cow as the random effect.

Somatic cell count was transformed by taking the Log_{10} and was analysed using the mixed effect model mentioned before. BCS data was analysed using Kruskal Wallis one way ANOVA at each period and also analysed to consider the change of BCS across periods e.g. P2 – P1, P3 – P2 and P4 – P3.

Values reported are least square means ± SEM and median for BCS. All statistical analyses were performed using R (R Core Team, 2013) with linear mixed effect analysis carried out using the nlme package (version 3.1-113). Statistical significance was taken as $P < 0.05$.

3.4 Trial 1

Yeast supplementation of housed commercial dairy cows under standard conditions

3.4.1 Introduction

Dairy feeding regimes are capable of altering the rumen environment by reducing daily rumen pH levels to thresholds that may result in subacute rumen acidosis (SARA). These feeding regimes face the challenge of providing an energetically high energy-density ration without compromising the animal's health, therefore the use of feed additives that will enhance health and performance by providing a stable rumen environment is common practice. It has been reported that the use of direct fed microbials may prevent a decline in rumen pH by altering the production and absorption of lactic acid (Fonty and Chaucheyras-Durand, 2006), alter feeding behaviour and improve rumination (Bach et al., 2007).

It is very seldom that different levels of yeast supplementation are evaluated. Wohlt et al. (1998) evaluated the addition of two doses of yeast at 10 or 20 g yeast / cow / d (50 or 100 x 10^9 cfu of *S. cerevisiae* respectively) to early lactation dairy cows. There were no statistically significant differences observed on the evaluated variables. However the authors reported a tendency for DMI, fat corrected milk yield (FCMY)

and acid detergent fibre digestibility to increase with the increment in yeast supplementation dose (Wohlt et al., 1998). Furthermore although the use of yeast is widespread in the dairy industry, no experimental reports have shown the effect of yeast on standard commercial practices. Furthermore it is paramount for the current Trial to validate the approach for yeast supplementation under standard farm environments. Therefore the aim of Trial 1 was to evaluate the effect that yeast supplementation at two contrasting doses had on cow performance, rumen pH and rumination time in loose housed commercial dairy cows consuming a standard commercial diet.

3.4.2 Material and methods

Trial 1was conducted during January – May 2012. All procedures related to animals were approved by the Veterinary Ethical Review Committee (Reference: VERC 2011-88) of the Royal (Dick) School of Veterinary Studies of the University of Edinburgh.

3.4.2.1 Animals and Housing

Fourteen multiparous milking cows were selected and balanced for DIM (mean ± SEM 104 ± 12 d) and parity [median lactation number (L) = 4]. The cows were then randomly allocated to two different Groups: Group 1 or Control (Cx: DIM = 103 ± 5 d, L = 5) and Group 2 or Treatment (Tx: 105 ± 5 d, L = 4), with seven cows in each Group. All cows were pregnant at the beginning of the Trial.

3.4.2.2 Experimental Design

The Control (Cx) group received no yeast supplementation. The Treatment (Tx) group received yeast supplementation at two levels. The Trial outline is presented in Table 3.2. Briefly the experiment was split into four Periods (P1, P2, P3 and P4), each Period lasted for three weeks. For each Period, cows had two weeks to adapt to

the diet and facilities, and all measurements were taken in the third week (Table 3.2). For example, in P1 cows were given two weeks to adapt to the diet and facilities and baseline measurements were recorded during the third week of P1.

3.4.2.3 Diets and yeast supplementation

Cows were offered a partial mixed ration (PMR) consisting of: first cut grass silage 46.2% (fresh weight PMR proportion), wholecrop wheat silage 18.0%, crimped maize 6.7% , dairy meal 24.1%, and molasses 5.1%) (Table 3.3).

The Tx Group received the same PMR and concentrate allocation as Cx with the addition of 0.8 g / cow / d (10 billion cfu/cow/d minimum recommended dose) yeast on P2 and 4.0 g/d (60 billion cfu/cow/d maximum recommended dose) of yeast were added for the three weeks of P3. In P4 the cows were offered the same PMR with no yeast supplementation ("wash-out" measurements) (Table 3.2). Cx group had no yeast supplementation throughout the Trial. The yeast was top-dressed on the PMR after fresh food was delivered at 0700 am. Cows were locked in the feed trough for 15 min using the self-locking neck yoke mechanism to ensure the cows ate all of the yeast supplementation.

Table 3.2 Timeline and experimental design used in Trial 1.

Experimental Periods												
	P1			P2			P3			P4		
Week	1	2	3	1	2	3	1	2	3	1	2	3
Week from	9th Jan	16th Jan	23rd Jan	30th Jan	6th Feb	13thFeb	20th Feb	27th Feb	5th Mar	12thMar	19thMar	26thMar
Cx = Control	PMR + concentrate			PMR + concentrate			PMR plus concentrate			PMR + concentrate		
Tx = Treatment	PMR + concentrate			PMR + concentrate+ 0.8 g/cow/d yeast			PMR + concentrate + 4.0 g/cow/d yeast			PMR + concentrate		

PMR = partial mixed ration.

3.4.2.4 Sampling procedures and measurements

Measurements were recorded on week 3 of each experimental period as detailed at the beginning of Chapter 3, following Table 3.1.

For rumen pH data collection, cows were orally administered an intra-rumen bolus (eCow Ltd., Devon, England UK). Prior to deployment each bolus was calibrated against known standard pH solutions (pH 4 and pH 7) (Osmotics, Aylsham, England UK). A bolus was administered to each cow using a bolus gun before the start of the first measurement week on P1.

3.4.2.5 Statistical Analysis

Statistical analysis was performed as discussed in section 3.3.5

3.4.3 Results

Table 3.3 shows the composition and chemical characteristics of the PMR fed throughout the Trial. The diet remained unchanged for the entire duration the Trial with no variation in the chemical characteristics of the PMR.

Table 3.3 Ingredients and chemical composition of the offered ration

Composition	Weight (kg / cow / day)		Periods			
	Fresh	DM	P 1	P 2	P 3	P 4
Ingredient						
Grass silage 1st cut	22	11				
Wholecrop wheat	8.80	4.3				
Langhill dairy meal	6.60	5.8				
Water	4.40	0.0				
Maize crimped	3.30	1.6				
Molasses	1.65	1.2				
Parlour concentrate (fed to yield)	3.0	2.63				
Analysis						
DM (%)			56.0	55.3	56.7	58.1
CP (% DM)			16.7	16.8	16.6	16.1
NDF (% DM)			35.0	33.0	33.0	35
uNDF forage (% DM)			6.9	6.4	7.6	7.6
uNDF total (% DM)			11.3	11.4	12.6	11.7
Oil (% DM)			3.9	4.0	3.9	3.9
Sugar (% DM)			9.6	9.5	9.7	9.5
Starch (% DM)			14.3	16.3	16.5	15.9
Quick CHO (% DM)			19.9	19.2	19.7	19.2
Slow CHO (% DM)			38.3	38.7	37.5	39.3

3.4.3.1 Milk yield and milk characteristics

Dietary yeast supplementation had no effect on milk yield or milk quality. Figure 3.1 shows the mean milk yield per group for the entire duration of the Trial, data is arranged by mean days in milk. Figure 3.2 shows the mean milk yield per group during the measurement week for each of the four Periods. No effect of treatment on mean milk yield was observed ($P > 0.05$).

Least square means for lactation performance are presented in Table 3.4. A statistically significant difference was observed (P<0.001) between experimental periods (P1, P2, P3 and P4: P<0.001), and a statistically significant effect of the interaction of Treatment X Period was observed for milk yield (P<0.05) (Table 3.5). An increase in milk yield was observed in the Tx Group when cows were fed the highest dose of yeast from P2 (mean = 36 kg milk) to P3 (mean = 41 kg milk), although the same tendency was observed for Cx group during P2 (mean = 38 kg milk) to P3 (mean = 39 kg milk) (Table 3.4).

No statistically significant differences between Cx and Tx group were observed in any of the milk composition characteristics. A Period effect was observed for all the variables analysed (P<0.001), no interaction with Treatment was observed (Table 3.4).

3.4.3.2 Body condition score and body weight

Yeast supplementation had no effect on BW, BCS or change in body condition score across the experimental periods (Table 3.4). The tendency of BCS and BW to decline (P1 to P3) and increase (P3 to P4) across Periods was similar and observed in both Groups (Table 3.4).

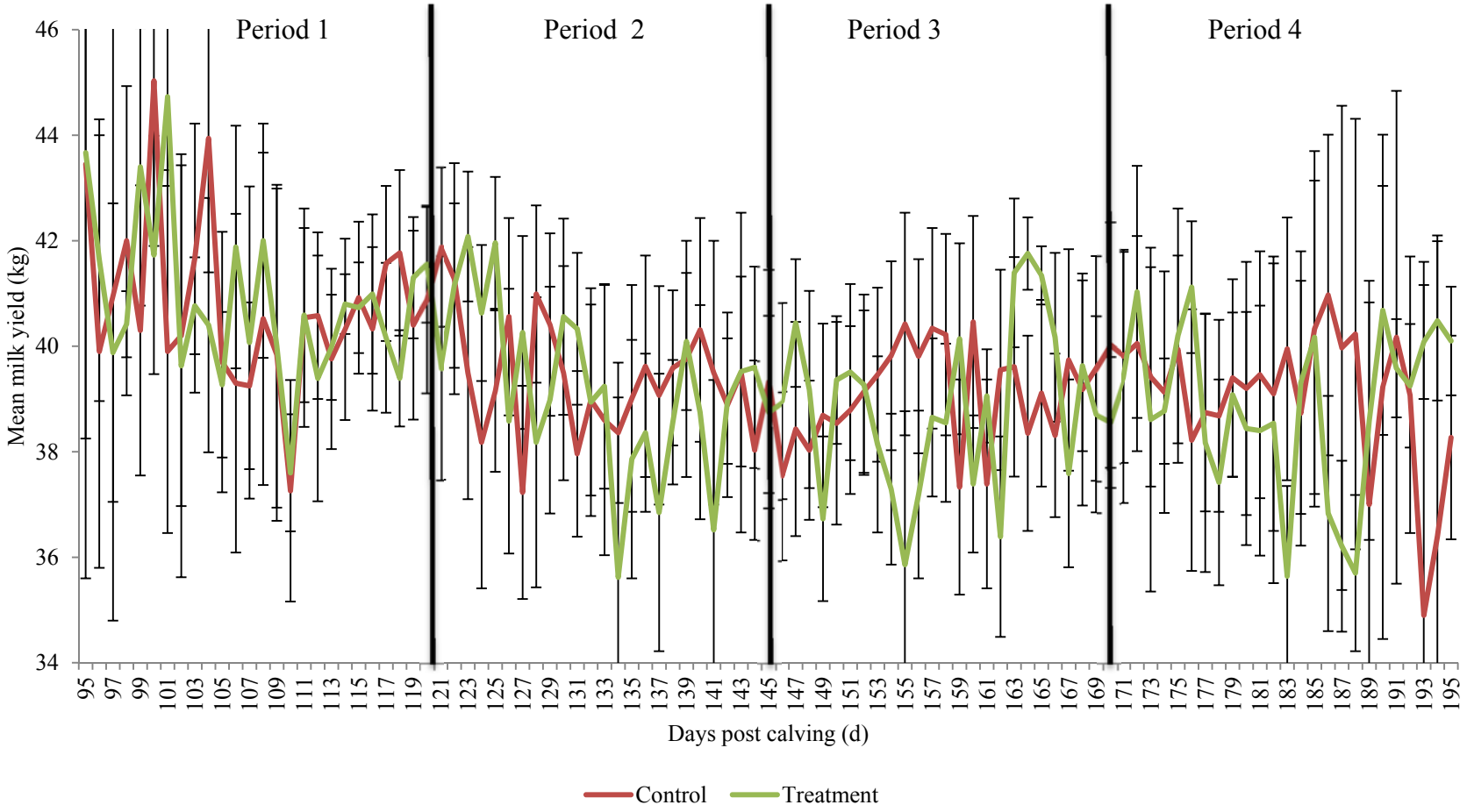

Figure 3.1 Group mean daily milk yield (± SEM) by mean days in milk recorded throughout the Trial. Group means were obtained from all the seven experimental animals of each group. Vertical solid black lines show the start and end of the different experimental periods.

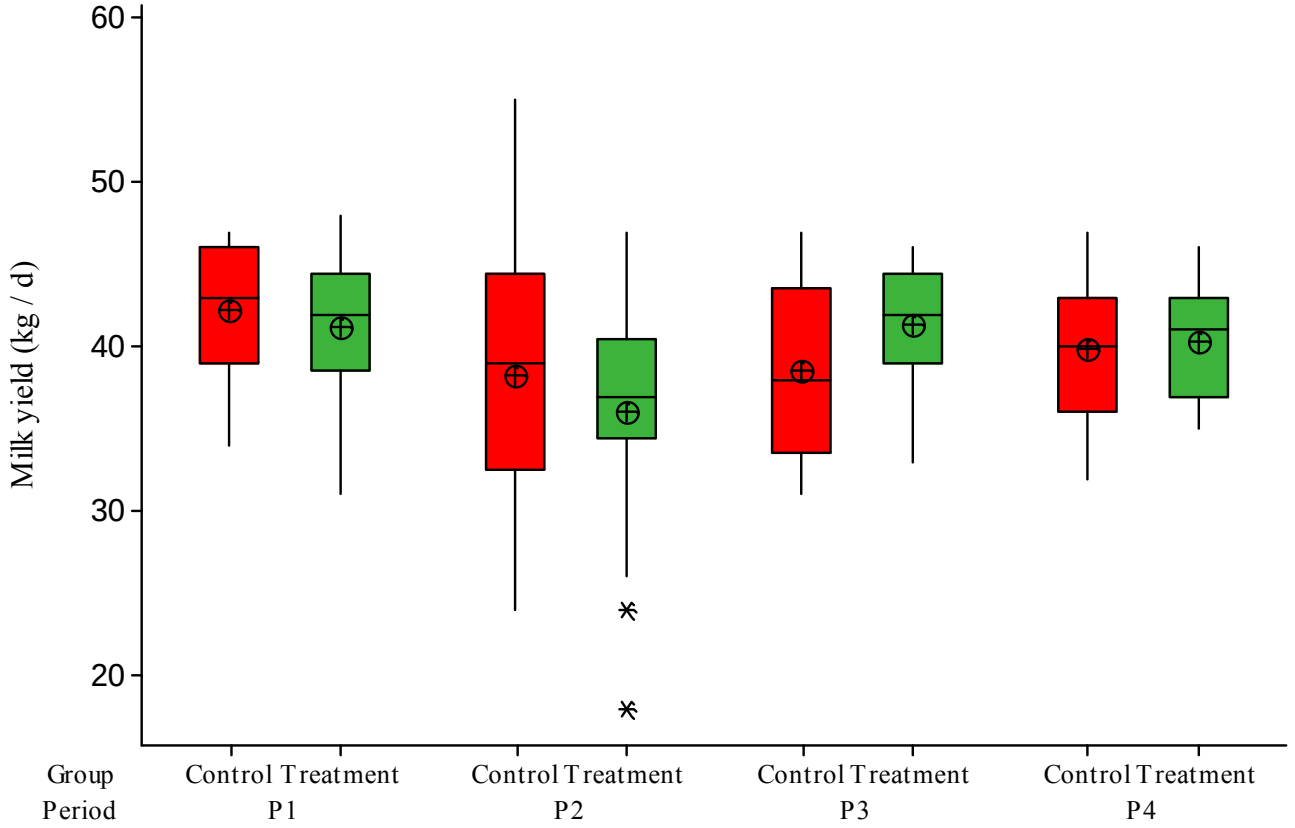

Figure 3.2 Group daily milk yield by Period. Data is presented as interquartile range (box) and median (horizontal line within the interquartile range), with asterisk representing outliers, circle with central cross representing means and vertical line (whiskers) representing the lower and upper 25% of the distribution. Red boxes represent Control group; green boxes represent Treatment group.

Table 3.4 Effect of yeast supplementation on milk yield and characteristics throughout the Trial 1

	Control				Treatment				P value		
Variable	Period 1	Period 2	Period3	Period 4	Period 1	Period 2	Period 3	Period 4	Period	Tx	Inter
Milk yield (kg/d)	42 ± 2	38 ± 2	39 ± 1	40 ± 1	41 ± 1	36 ± 1	41 ± 1	40 ± 1	<0.05	0.99	0.04
Butter fat (%)	4.23 ± 0.23	3.66 ± 0.23	3.56 ± 0.23	3.75 ± 0.23	4.22 ± 0.23	3.88 ± 0.23	3.93 ± 0.23	3.68 ± 0.23	<0.05	0.68	0.08
Protein (%)	3.30 ±0.08	3.29 ± 0.08	3.35 ± 0.08	3.34 ± 0.08	3.29 ± 0.08	3.26 ± 0.08	3.25 ± 0.08	3.33 ± 0.08	<0.05	0.74	0.06
Lactose (%)	4.55 ± 0.03	4.50 ± 0.03	4.52 ± 0.03	4.54 ± 0.03	4.53 ± 0.03	4.49 ± 0.03	4.44 ± 0.03	4.52 ± 0.03	<0.05	0.42	0.20
SCC log_{10}	1.51 ± 0.21	1.52 ± 0.21	1.53 ± 0.21	1.70 ± 0.21	1.66 ± 0.21	1.52 ± 0.21	1.58 ± 0.21	1.92 ± 0.21	<0.05	0.72	0.33
BW (kg)	711 ± 22	701 ± 22	704 ± 22	710 ±22	668 ± 22	659 ± 22	667 ± 22	674 ± 22	<0.05	0.23	0.85
BCS analyses											
BCS (1 – 5)	2.75	2.50	1.75	2.25	2.75	2.50	2.00	2.25			
	P2 – P1	P3 – P2	P4 – P3		P2 – P1	P3 – P2	P4 – P3				
BCS Change	-0.25	-0.25	0.25		-0.25	-0.25	-0.5			0.43	

Mean ± SEM are presented for milk yield, milk characteristics and BW. BCS is presented as median. Inter = interaction

3.4.3.3 Rumination time

No effect of yeast supplementation was observed on the amount of time (min /d) the cows spent ruminating. An effect of Period on rumination time was observed ($P<0.05$), however this effect was the same for both Groups (Table 3.5).

3.4.3.3 Rumen pH

Figure 3.3 shows an example of the rumen pH circadian pattern observed in two of the experimental cows, one from each Group in Period 1. Reliable data on rumen pH was obtained from the rumen boluses, however incomplete datasets were obtained from some of the animals and boluses were lost across the Trial. Complete data on rumen pH values per hour were obtained from 14 cows in P1, and 11 cows in P2 and P3, and only 4 cows in P4. Therefore it was decided not to analyse data from P4 due to an insufficient number of recordings. Data on rumen pH variables is therefore only presented for P1 to P3.

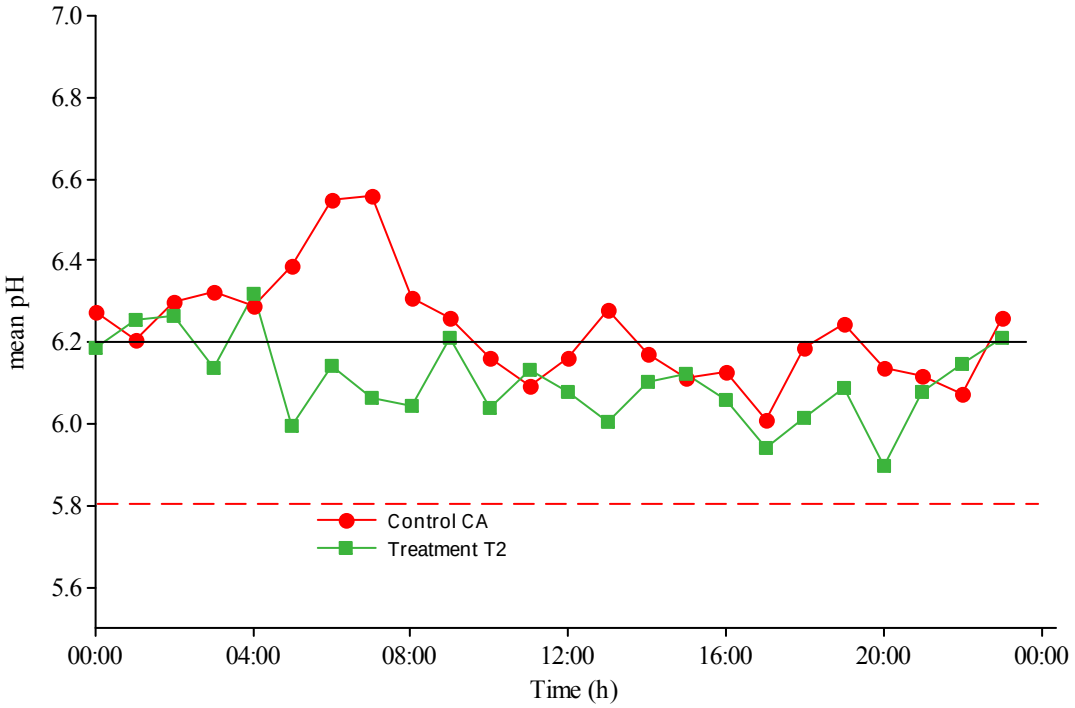

Figure 3.3 Diurnal pH dynamic of two cows (one cow per Group)

No differences were observed in mean rumen pH values between Cx and Tx Groups, however an effect of Period was observed ($P<0.05$) (Table 3.5). Mean rumen pH tended to decline across the experimental periods. When analysing the dynamics of rumen pH, different values of time spent under the acidotic thresholds were observed. As with the mean pH, cows tended to spend more time under the acidotic thresholds across the experimental periods. In both experimental groups when rumen pH dynamics was analysed as time spent below pH 6.2, an effect of period was observed ($P<0.005$) (Table 3.5), when the time spent under the pH 6.2 threshold tended to increase (Figure 3.4). However no effect of yeast supplementation was observed (Table 3.5). No effect of yeast supplementation was observed when time spent under SARA conditions was determined using a threshold of pH < 5.8 (Table 3.5 and Figure 3.5).

Table 3.5 Effect of yeast supplementation on rumen pH and rumination time throughout the Trial.

	Control				Treatment				P value		
Variable	Period 1	Period 2	Period 3	Period 4	Period 1	Period 2	Period 3	Period 4	Period	Tx	Inter
Rumen pH											
Mean pH	6.38 ± 0.05	6.30 ± 0.05	6.22 ± 0.05	NA	6.43 ± 0.07	6.34 ± 0.07	6.24 ± 0.07	NA	<0.01	0.67	0.15
Time pH <6.2 (min/d)	291 ± 114	439 ± 114	589 ± 114	NA	218 ± 151	405 ± 151	634 ± 151	NA	<0.01	0.89	0.79
Time pH <5.8 (min/d)	9 ± 12	6 ± 12	3 ± 12		4 ± 16	9 ± 16	7 ± 16		0.44	0.34	0.87
Rumination											
Time (min/d)	495 ± 23	512 ± 22	480 ± 23	504 ± 23	526 ± 23	551 ± 22	528 ± 23	536 ± 23	<0.05	0.25	<0.78

Mean ± SEM. Tx = treatment, Inter = interaction

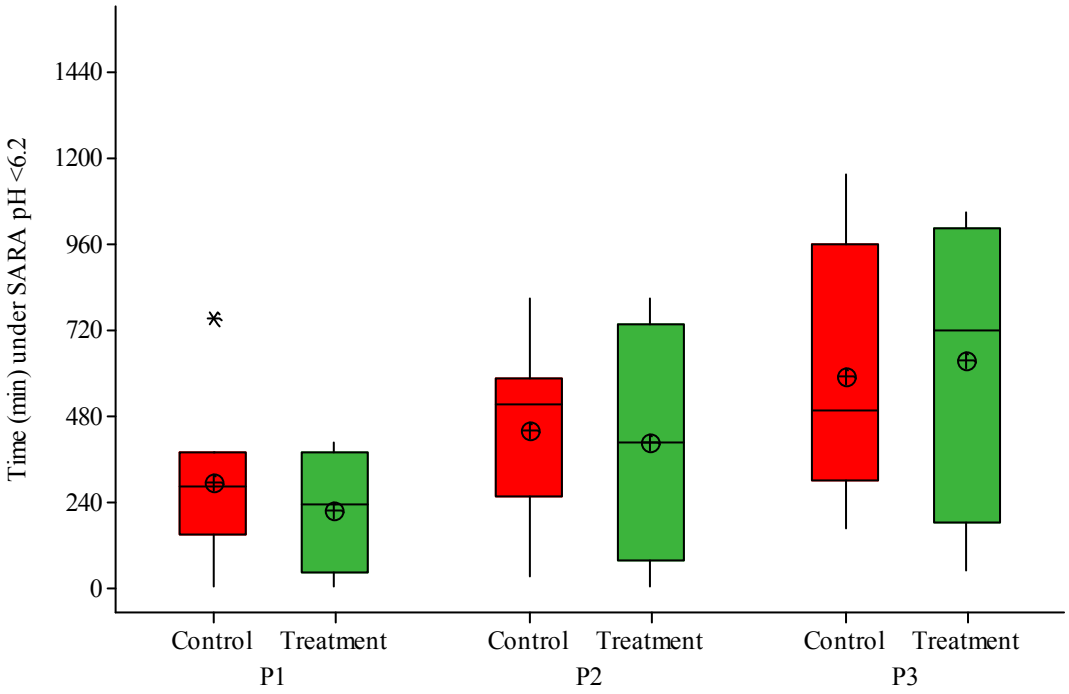

Figure 3.4 Time spent under SARA (pH<6.2) per Group and Period. Data is presented as interquartile range (box) and median (horizontal line within the interquartile range), with asterisk representing outliers, circle with central cross representing means and vertical line (whiskers) representing the lower and upper 25% of the distribution. Red boxes represent Control group whereas green boxes represent Treatment group.

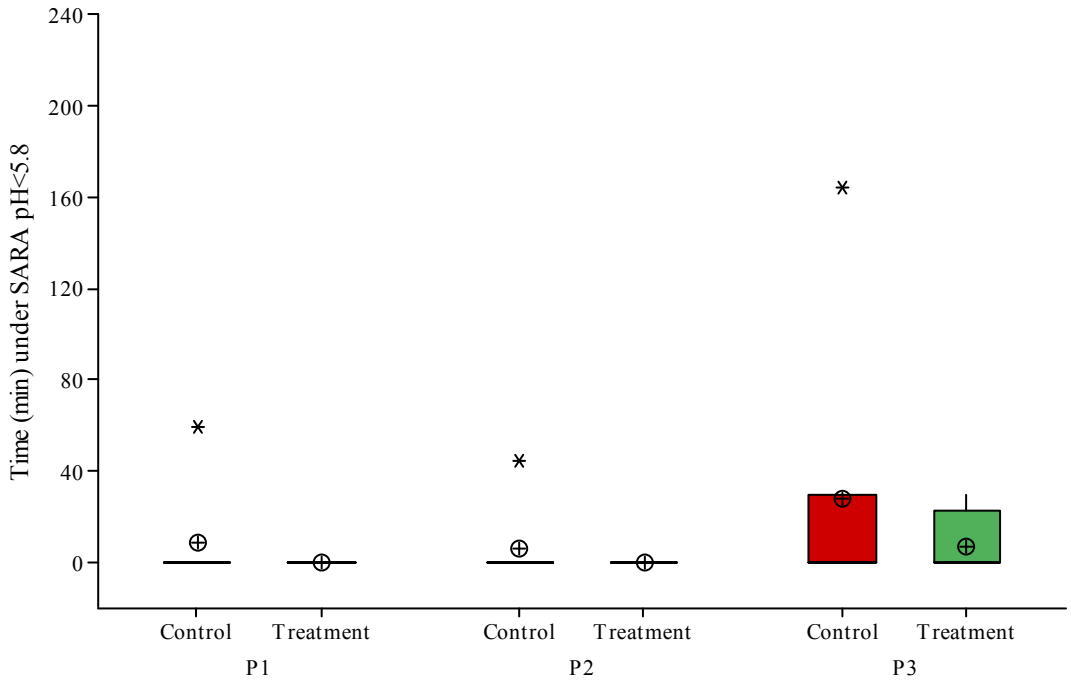

Figure 3.5 Time spent under SARA (pH<5.8) per Group and Period. Data is presented as interquartile range (box) and median (horizontal line within the interquartile range), with asterisk representing outliers, circle with central cross representing means and vertical line (whiskers) representing the lower and upper 25% of the distribution. Red bars represent Control group whereas green bars represent Treatment group.

3.4.4 Discussion

The aim of Trial 1 was to evaluate the effect of yeast supplementation on performance, rumination time and rumen pH in cows fed a standard commercial diet. No statistically significant differences were observed on any of the parameters measured between Cx and Tx Groups. These results are in line with previous studies that found no statistically significant difference for production parameters or performance between cows receiving yeast supplementation compared with those on a control diet. Kalmus et al. (2009) found no effect of yeast supplementation on milk yield, while Al Ibrahim et al. (2010) found no effect on milk yield, milk fat, protein and lactose content either. Similarly Moallem et al. (2009) reported no statistically significant difference between cows fed yeast against those that were not supplemented in terms of milk fat and milk protein. Kung et al. (1997) found no effect of yeast supplementation on milk yield of mid lactation dairy cows. Similarly when evaluating the effect of yeast cultures on lactating performance of early lactation dairy cows, Swartz et al. (1994) found no effect when compared with cows with no yeast supplementation.

Results on the effect of yeast supplementation on BCS and BW are very seldom reported. However Kalmus et al. (2009) and Al Ibrahim et al. (2010) found no effect on BCS when yeast was supplemented to cows in early lactation.

No differences were observed between the different doses of yeast fed. However slight increments on milk yield and butterfat were observed when the highest dose of yeast was supplemented (50 x 10^9 cfu / cow / d). These results are similar to those reported by Wohlt et al. (1998) that reported better responses on milk yield and FCMY when 100 x 10^9 cfu / cow / d were fed compared with the lower dose of 50 x 10^9 cfu / cow / d fed in their trial. Similarly Zaworski et al. (2014) found differences when feeding increasing levels of inclusion (56 or 112g) of yeast fermentation product to early lactation multiparous dairy cows. Cows fed the yeast fermentation product tended to increase milk yield, however the increment was not dose dependant.

Contrasting results however were reported by Ferraretto et al. (2012) when they fed mid lactation dairy cows consuming contrasting diets, two different doses of yeast (30 or 60 x 10^9 cfu / cow / d). The authors found no effect ($P > 0.05$) of yeast supplementation in any of the variables studied, although differences between the two doses supplemented were observed, total NDF digestibility and milk fat content was higher when 60 x 109 cfu / cow / d was administered.

The development of automated methods to record cow behaviour facilitated the evaluation of the effect that yeast supplementation may have on rumination time. The RC has enabled research related to rumination activity, that looks into the relationship that rumination might have with different events across the lactation cycle e.g. trying to relate rumination activity with calving (Clark et al., 2015). In this area, a recent study carried out by Devries and Chevaux (2014) looked to investigate the effect of yeast supplementation on feeding behaviour of dairy cows. The authors found that yeast supplementation had no effect on meal pattern or rumination time, however they reported a tendency for rumination time to increase with yeast supplementation. These results are in line with our findings that rumination time in the Tx group tended to increase across the Periods (Table 3.5). When looking at the relationship of rumination time and SARA, a study by DeVries et al. (2009) reported a reduction in rumination time (min / d) across periods when cows were fed an acidogenic diet (DeVries et al., 2009). In line with this result, it was observed that cows in Trial 1 that had longer episodes of SARA tended to spent less time ruminating. This was evident in Cx group, however this was not observed for the Tx Group.

Research on the effect of yeast supplementation on rumen environment (e.g. rumen pH) has been carried out *in vitro* and *in vivo*, by using fistulated animals or by taking rumen fluid samples using rumenocentesis in intact animals. The advent of the rumen pH bolus means that studies with intact animals under commercial farm conditions can be carried out. Nevertheless using cannulated dairy cows in late lactation, Thrune et al. (2009) found that yeast supplementation had an effect on rumen pH. In their study, yeast fed animals had higher mean rumen pH, and spent less time under the acidotic thresholds (pH < 6.2, 6.0 and 5.8) when compared to those on a control diet.

Al Ibrahim et al. (2010) analysed the rumen fluid of dairy cows in early lactation obtained by rumenocentesis, and they reported no statistically significant differences on rumen pH and other rumen fermentation parameters in yeast supplemented cows. In a similar study (using rumen samples obtained via rumenocentesis from early lactation cows), Moallem et al. (2009) reported comparable findings with mean rumen pH not affected by yeast supplementation.

Our results showed an effect of period (or time) on rumen pH, with decreasing values of mean rumen pH and increasing time (min / d) of rumen pH spent under SARA thresholds. These findings are difficult to compare with other studies that used more invasive techniques (such as fistulated cows or rumenocentesis). By their nature, these more invasive techniques mean that the length of the period evaluated is shorter, and data sets obtained are smaller. For example Thrune et al. (2009) recorded rumen pH for only 6 d and other studies using rumenocentesis only reported one time point a day. This makes their data more prone to variation, and lacking in statistical power.

The observed effect of period could be explained by the stage of lactation the cows were at throughout Trial 1 (mid to late lactation). A normal reduction in milk yield is expected after cows reach their peak milk yield; this reduction in milk production could affect intakes, which in turn affect pH dynamics hence less time the cows spent under SARA.

Furthermore it is noteworthy that although no statistically significant differences between milk yields were observed, an arithmetical difference of more than 1 kg milk between Groups was observed. In Period 3 when cows in the Tx Group were fed the highest dose of yeast, a larger difference in milk yield was observed (Cx = 39 kg and Tx = 41 kg). These results are similar to those obtained for cows in early (Erasmus et al., 2005; Kung et al., 1997; Nocek et al., 2011; Robinson and Garrett, 1999; Williams et al., 1991; Wohlt et al., 1998) or mid to late lactation cows (Moallem et al., 2009; Schingoethe et al., 2004) supplemented with live yeast or yeast culture.

In the present study, yeast supplementation had no effect on any of the recorded variables, and no conclusive evidence on the benefits of yeast supplementation was found. It might be that studies on animals at different stages of lactation, consuming more challenging diets, or in different environments such as grazing might show a difference when the rumen environment is under greater challenge.

3.5 Trial 2

Yeast supplementation in commercial dairy cows with induced bouts of SARA

3.5.1 Introduction

SARA is a digestive disorder characterized by low levels of rumen pH (<5.8) caused either by lack of structural fibre or an excess of concentrate, generally rapidly fermentable carbohydrates. SARA is commonly present in dairy cows and can cause erratic or depressed feed intake, diarrhoea, decreased milk yield, and low milk fat content. Dairy managers and nutritionists have used different feeding strategies and the use of feed additives to eradicate, control or prevent the occurrence of SARA. The inclusion of high effective fibre material in the diet is advisable, and the use of supplementary bicarbonate, yeast and yeast cultures are also common practices in dairy cow nutrition.

However as mentioned earlier in Chapter 3, the results obtained with yeast supplementation are contentious. It is thought that yeast enables the stabilisation of rumen pH by: a) promotion of lactic acid utilization, b) O_2 consumption, c) competition with rumen micro-organism for available sources of energy and d) providing growth factors (Newbold et al., 1996). By affecting rumen ecology it is proposed that *Saccharomyces cerevisiae* could influence rumen pH. *In vitro* studies have reported that live yeast culture could influence pH level by altering the balance of lactate metabolising bacteria and favour the uptake of lactate by microorganisms such as *Megasphaera elsdenii* (Rossi et al., 2004). Brossard et al. (2006) reported that one strain of *S cerevisiae* could prevent decreases in rumen pH by stimulating certain populations of ciliate protozoa, which rapidly engulf starch and thereby effectively compete with amylolytic, lactate producing bacteria (*Enterococcus, Lactobacillus spp.*). This would sustain a constant level of lactic acid, thus allowing the lactate utilising species to flourish and so present a possible means to limit acidosis in high concentrate fed animals (Brossard et al., 2006; Nocek and Kautz, 2006). Furthermore some studies suggested that the effect of yeast is more evident

when the host faces environmental, management or physiological challenges that might increase the incidence of SARA. Salvati et al. (2015) reported that yeast supplementation improved lactation performance of dairy cows under heat stress. Therefore the aim of Trial 2 was to evaluate the effect that yeast supplementation had on cow performance, rumen pH and rumination time in loose housed commercial dairy cows consuming an acidogenic diet.

3.5.2 Materials and methods

Trial 2 was conducted during January – May 2013. All procedures related to animals were approved by the Veterinary Ethical Review Committee (Reference: VERC 30 / 12) of the Royal (Dick) School of Veterinary Studies of the University of Edinburgh. Trial 2 had the same overall structure as Trial 1, and so only specific details and differences are provided.

3.5.2.1 Animals and Housing

Fourteen multiparous milking cows were selected and balanced for DIM (97 ± 4.3d) and parity (L = 3). The cows were then randomly allocated to 2 different Groups: G1: (DIM = 96 ± 2.7 and L = 3) and G2: (DIM = 99 ± 9.2 d, L = 4), with 7 cows in each group. All cows but two were pregnant at the beginning of the Trial.

3.5.2.2 Experimental Design

Cows were divided into two groups G1 and G2. The experiment was split into four Periods (P1, P2, P3 and P4: P1 (baseline), P2 and P3 (Treatment) and P4 (washout). In Period 1, the cows had two weeks to adapt to the facilities and diets and all measurements were taken on week three. For each of the remaining Periods (P2, P3 and P4) cows had three weeks to adapt to the diet and facilities, and all measurements were taken in the fourth week (Table 3.6). In P2 and P3 yeast

supplementation at 4.0 g / cow / d (60 billion cfu / cow / d) followed a cross-over experimental design.

Table 3.6 Experimental time scale of Trial 2

	Experimental Periods															
	Period 1			Period 2				Period 3				Period 4				
Week	1	2	3	1	2	3	4 Obs	1	2	3	4 Obs	1	2	3	4 Obs	
Week commencing	14th Jan	21st Jan	28th Jan	4th Feb	11th Feb	18th Feb	25th Feb	4th Mar	11th Mar	18th Mar	25th Mar	1st Apr	8th Apr	15th Apr	22nd Apr	
Group 1	PMR + Concentrate + 1.5kg ground wheat / cow / day			**PMR + Concentrate + 1.5kg ground wheat / cow / day + 4.0 g yeast / cow / day**				PMR + Concentrate + 1.5kg ground wheat / cow / day				PMR + Concentrate + 1.5kg ground wheat / cow / day				
Group 2	PMR + Concentrate + 1.5kg ground wheat / cow / day			PMR + Concentrate + 1.5kg ground wheat / cow / day				**PMR + Concentrate + 1.5kg ground wheat / cow / day + 4.0 g yeast / cow / day**				PMR + Concentrate + 1.5kg ground wheat / cow / day				

3.5.2.3 Diets, yeast supplementation and induction of bouts of acidosis

Cows were offered a PMR (first cut grass silage 44.9%, wholecrop wheat silage 17.6%, second cut grass silage 15.6%, dairy meal 18.5% and molasses 3.4%), with additional concentrate fed to yield in the milking parlour. Additionally 1.5 kg per cow per day of ground wheat was added to the PMR to induce bouts of SARA. After fresh food was delivered at approximately 0700, the wheat was added to the feed and mixed until homogeneity was obtained. Yeast was supplemented on P2 and P3 following a cross-over design. Yeast was supplemented at 4.0 g cow / d (50 billion cfu/cow/day) in P2 and P3 (Table 3.6). The yeast was top-dressed on the PMR after fresh food was delivered at 0700. Cows were locked in the feed trough for 15 min using the self-locking neck yoke mechanism to ensure the cows ate all of the yeast supplemented.

3.5.2.4 Sampling procedures and measurements

All measurements were recorded on the last week of each experimental Period (P1 = week 3 and P2, P3 and P4 = week 4) as described in Section 3.3.4 and Table 3.1.

Rumen pH was recorded using a rumen bolus (WellCow Ltd., Roslin, Scotland UK). Prior to deployment each bolus was calibrated against known standard pH solutions (pH 4 and pH 7 provided by WellCow Ltd).

3.5.2.5 Statistical Analysis

Statistical analysis was performed as detailed in Section 3.3.5. The linear mixed effect model used to analyse all the variables included fixed effects of period and treatment, and the interaction of period and treatment with cow as the random effect. The effect of sequence of yeast supplementation (Treatment to Control vs Control to Treatment) was also included in the model.

3.5.3 Results

Table 3.7 shows the composition and chemical characteristics of the PMR fed, and the PMR remained the same throughout the Trial.

Table 3.7 Ingredients and chemical composition of the offered ration

Composition	Weight (kg / cow /day)		Periods			
	Fresh	DM	P 1	P 2	P 3	P 4
Ingredient						
Grass silage 1st cut	23.0	5.75				
Grass silage 2nd cut	8	1.86				
Wholecrop wheat	9.00	3.82				
Langhill dairy meal	9.5	8.46				
Molasses	1.75	1.31				
Wheat (group)	1.5	1.20				
Parlour concentrate (fed to yield)	3.0	2.63				
Analysis						
DM (%)			40.6	40.8	41.9	42.3
CP (% DM)			16.7	16.2	16.2	16.6
NDF (% DM)			35	36	34	36
uNDF forage (% DM)			6.5	6.8	6.3	6.5
uNDF total (% DM)			10.3	10.7	9.9	10.2
Oil (% DM)			3.1	3.2	3.4	3.0
Sugar (% DM)			7.1	7.5	7.6	7.2
Starch (% DM)			19.8	19.8	19.2	19.1
Quick CHO (% DM)			15.6	15.8	16.4	16
Slow CHO (% DM)			42.2	43	41.9	42

3.5.3.1 Milk yield and milk characteristics

Figure 3.6 shows the mean milk yield per experimental Group for the entire duration of the Trial with results arranged by mean days in milk. Figure 3.7 presents the mean milk yield per Group during the measurement week only, for each of the four Periods. No effect of treatment on mean milk yield in the experimental Groups was observed. A statistically significant effect of Period was observed ($P<0.001$) with no interaction Treatment X Period observed (Table 3.8).

No effect of yeast supplementation on any of the milk composition characteristics was observed. A Period effect was observed for all the variables analysed ($P<0.001$). However no interaction Treatment X Period was observed (Table 3.8).

3.5.3.2 Body condition score and body weight

Yeast supplementation had no effect on BW, BCS or change in body condition score across the experimental periods (Table 3.8). A tendency for BCS to decline (P1 to P3) and increase (P3 to P4) was observed in both experimental Groups (Table 3.8).

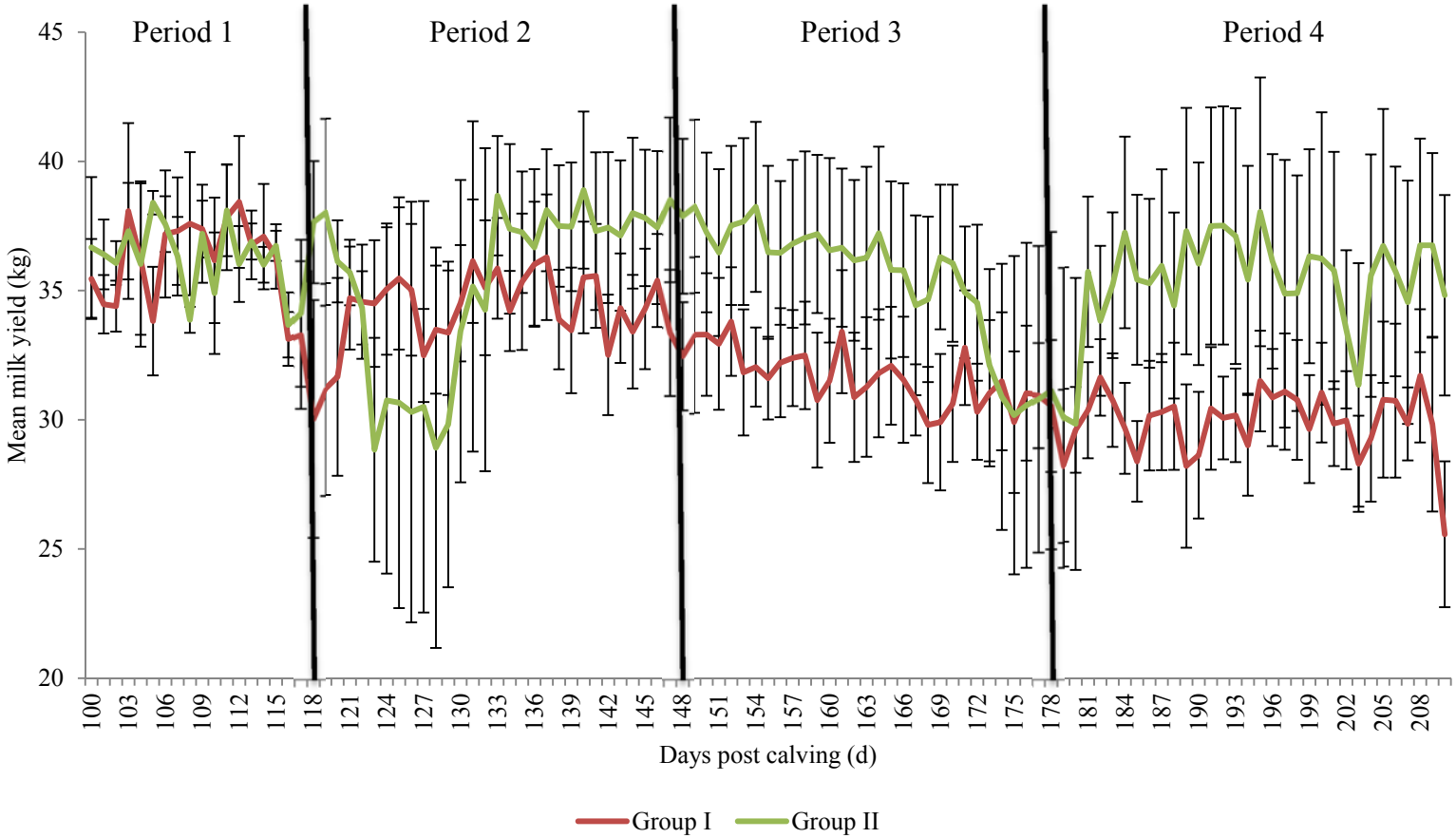

Figure 3.6 Group mean daily milk yield (± SEM) by mean days in milk recorded throughout the Trial. Group means were obtained from seven experimental animals in each group. The vertical solid black lines represent the start and end of each experimental period.

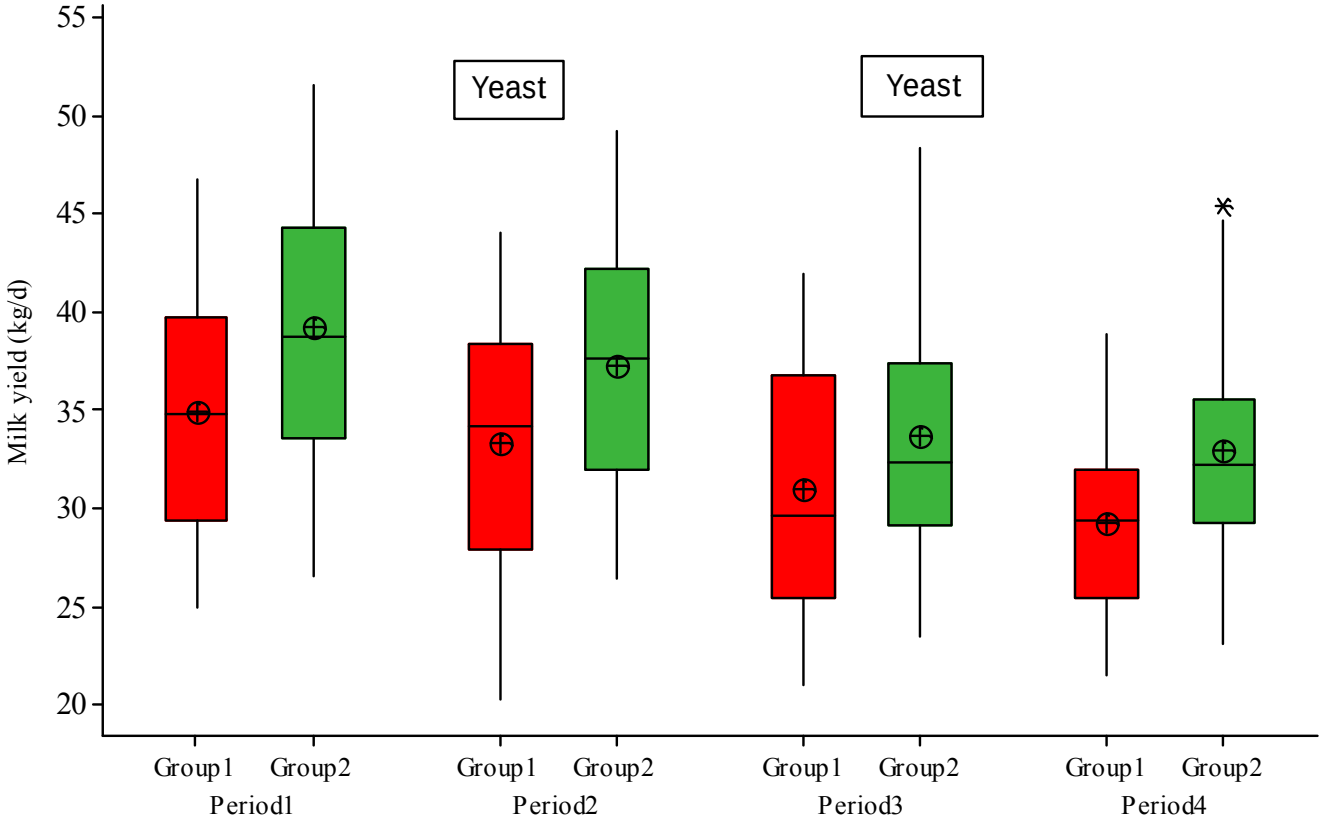

Figure 3.7 Group daily milk yield by Period. Data is presented as interquartile range (box) and median (horizontal line within the interquartile range), with asterisk representing outliers, circle with central cross representing means and vertical line (whiskers) representing the lower and upper 25% of the distribution. Red boxes represent Group 1 and green boxes represent Group 2.

Table 3.8 Effect of yeast supplementation on milk yield and characteristics throughout Trial 2

Variable	Group 1				Group 2				P value		
	Period 1	Period 2	Period 3	Period 4	Period 1	Period 2	Period 3	Period 4	Period	Tx	Interaction
		Yeast					**Yeast**				
Milk yield (kg/d)	35 ± 2.13	33 ± 2.13	31 ± 2.13	29 ± 2.13	39 ± 2.13	37 ± 2.13	34 ± 2.13	33 ± 2.13	<0.001	0.23	0.30
Butter fat (%)	4.6 ± 2	4.37 ± 0.27	4.72 ± 0.27	4.54 ± 0.27	4.63 ± 0.27	4.49 ± 0.27	4.80 ± 0.27	4.73 ± 0.27	<0.05	0.77	0.87
Protein (%)	3.41 ± 0.12	3.45 ± 0.12	3.56 ± 0.12	3.47 ± 0.12	3.34 ± 0.12	3.43 ± 0.12	3.58 ± 0.12	3.42 ± 0.12	<0.005	0.85	0.33
Lactose (%)	4.51 ± 0.05	4.44 ± 0.05	4.48 ± 0.05	4.33 ± 0.05	4.55 ± 0.05	4.50 ± 0.05	4.45 ± 0.05	4.33 ± 0.05	<0.005	0.75	0.20
BW (kg)	684 ± 12	684 ± 12	685 ± 12	695 ± 12	701 ± 12	704 ± 12	700 ± 12	707 ± 12	<0.01	0.35	0.44
BCS analyses											
BCS (1 – 5)	2.75	2.50	1.75	2.25	2.75	2.50	2.00	2.25			
	P2 – P1	P3 – P2	P4 – P3		P2 – P1	P3 – P2	P4 – P3				
BCS Change	0	-0.5	-0.25		0	-0.25	0				

Means ± SEM are presented for milk yield, milk characteristics and BW. BCS is presented as median. Tx = treatment

3.5.3.3 Rumination time

No effect of yeast supplementation on the amount of time (min/d) the cows spent ruminating was observed. An effect of period on rumination time was observed ($P<0.05$) (Table 3.9).

3.5.3.3 Rumen pH

Reliable data was obtained from the rumen boluses (Figure 3.8). Although individual cow response to the acidotic challenge of 1.5 kg of ground wheat was variable, the supplementation of 1.5 kg ground wheat / cow / day was able to produce considerable bouts of acidosis (Figure 3.9). Complete data on rumen pH values per hour were obtained from 10 cows in P1, P2 and P3 and only for 3 cows in P4. Therefore it was decided not to analyse any of this data from P4, and so data on rumen pH variables is presented for P1 to P3 only.

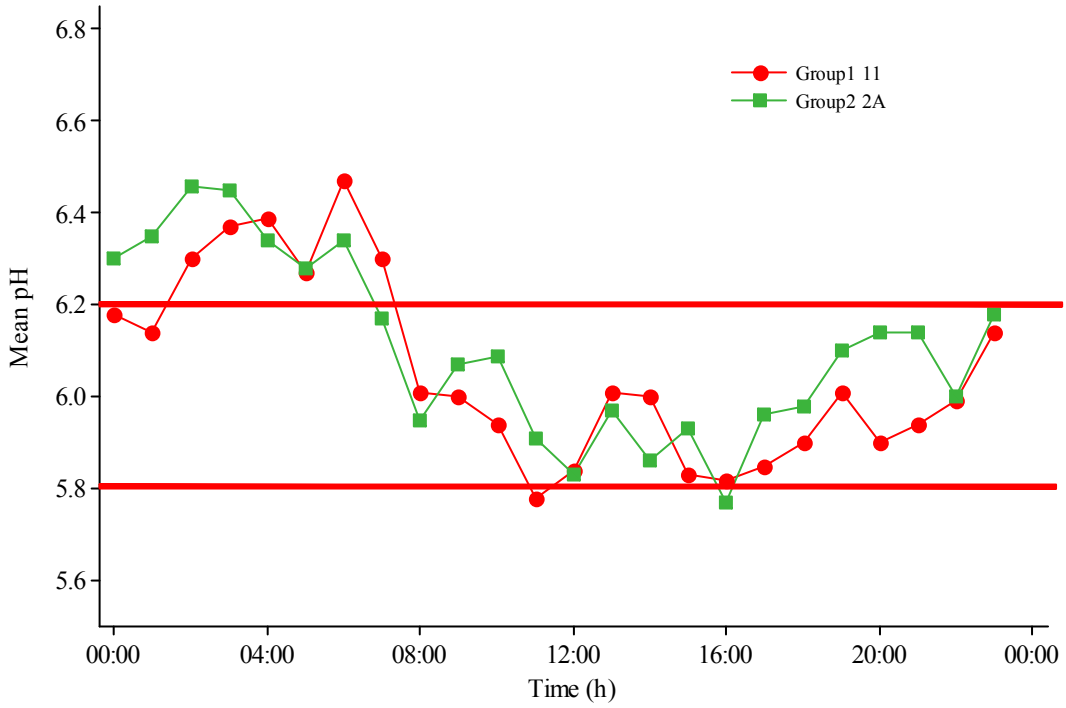

Figure 3.8 Circadian pH dynamics of two cows (one for each Group). The red line shows the 6.2 and 5.8 pH threshold used as a cut-off point for SARA diagnosis. Both cows spent more than 10 h a day below this threshold after the acidotic challenge i.e. addition of 1.5 kg / cow / day of group wheat to the PMR at around 07:00.

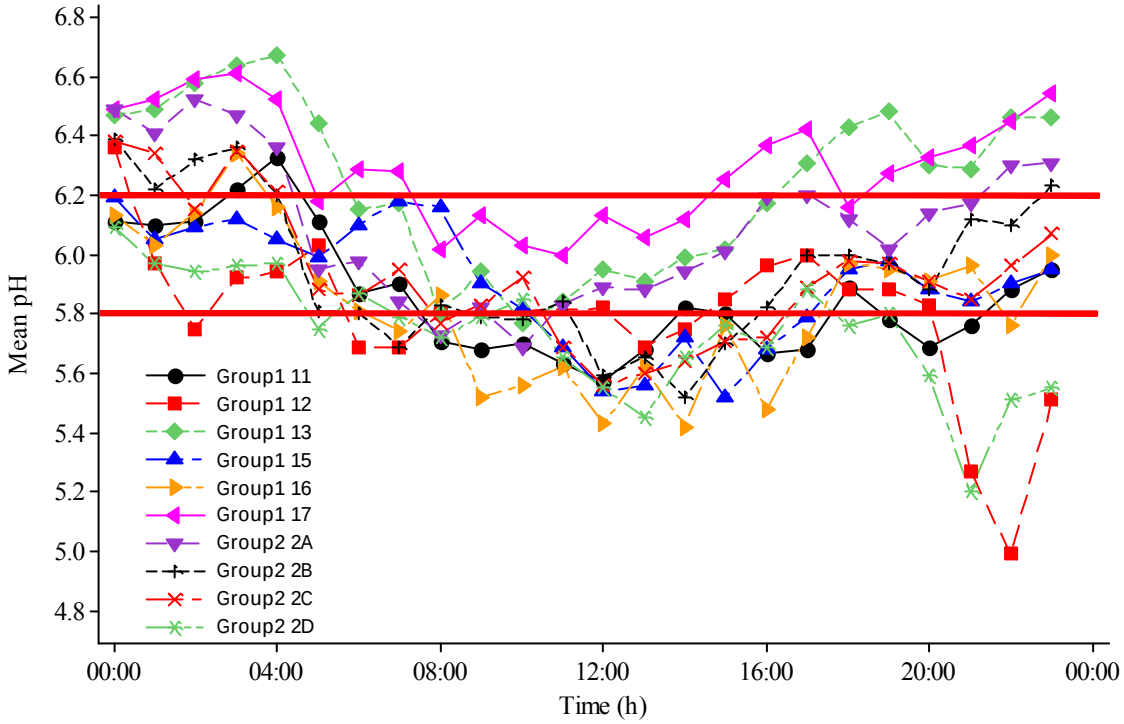

Figure 3.9 Circadian pH dynamics of 10 cows from both Groups. The red line shows the 6.2 and 5.8 rumen pH SARA threshold. Addition of 1.5 kg / cow / day of ground wheat to the PMR at around 07:00

No differences were observed on mean rumen pH values in yeast supplemented versus non-supplemented animals (Table 3.9). However an effect of Period was observed ($P<0.05$). Mean rumen pH tended to decline across the experimental periods. When analysing the dynamics of rumen pH, different periods of time spent under the acidotic thresholds were observed. As with the mean pH values, the cows tended to spend more time under the acidotic thresholds across the experimental periods. An effect of period was observed ($P<0.005$) when time spent below pH 6.2 was analysed, although no effect of yeast supplementation was observed. Similar results were observed when time spent below pH 5.8 was considered, with no effect of yeast supplementation observed. Again there was a significant effect of period present. No effect of the sequence followed by yeast supplementation was observed on any of the rumen pH variables analysed.

Table 3.9 Effect of yeast supplementation on rumen pH and rumination time throughout Trial 2.

Variable	Group 1				Group 2				P value		
	Period 1	Period 2	Period 3	Period 4	Period 1	Period 2	Period 3	Period 4	Period	Tx	Interaction
		Yeast					**Yeast**				
Rumen pH											
Mean pH	6.14 ± 0.06	5.99 ± 0.06	5.88 ± 0.06	NA	6.18 ± 0.06	5.93 ± 0.07	5.86 ± 0.07	NA	<0.001	0.86	0.05
Time pH <6.2 (min/d)	840 ± 121	1040 ± 120	1228 ± 120	NA	743 ± 148	1178 ± 148	1264 ± 147	NA	<0.001	0.88	0.42
Time pH <5.8 (min/d)	118 ± 123	418 ± 123	625 ± 123		30 ± 151	503 ± 151	664 ± 151		<0.001	0.93	0.70
Rumination											
Time (m/d)	406 ± 25	399 ± 25	361 ± 25	400 ± 26	435 ± 25	481 ± 25	472 ± 25	503 ± 27	<0.01	0.05	<0.001

Mean ± SEM. Tx = treatment

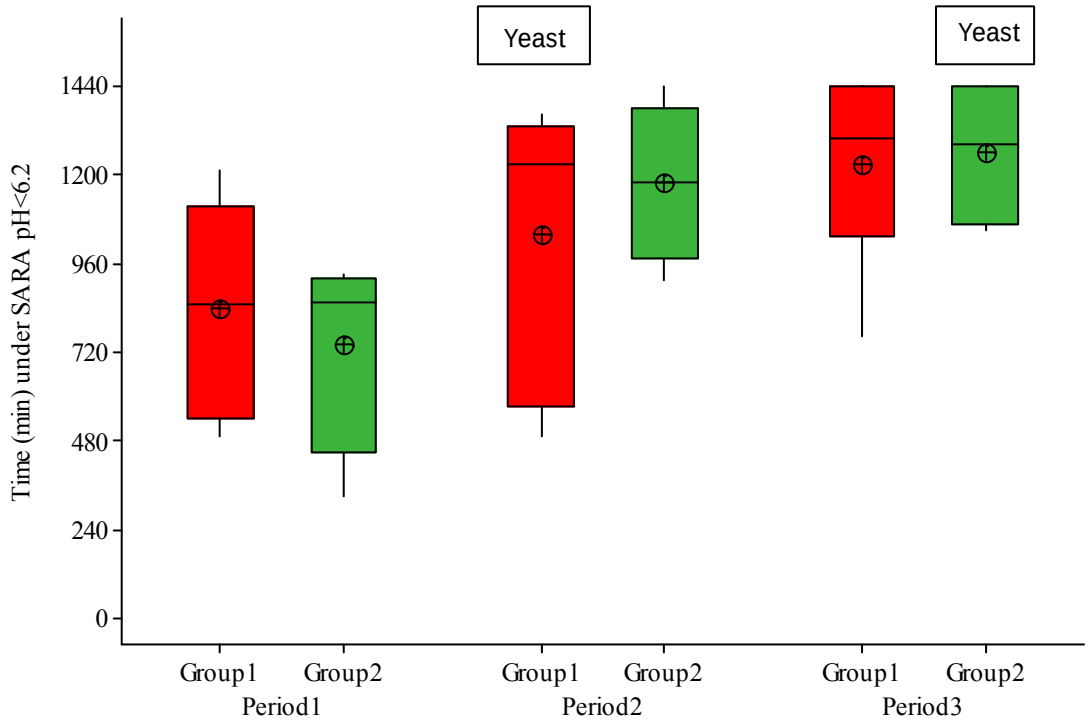

Figure 3.10 Time spent under SARA (pH<6.2) per Group and Period. Data is presented as interquartile range (box) and median (horizontal line within the interquartile range), with asterisk representing outliers, circle with central cross representing means and vertical line (whiskers) representing the lower and upper 25% of the distribution. Red boxes represent Group 1 while green boxes represent Group 2.

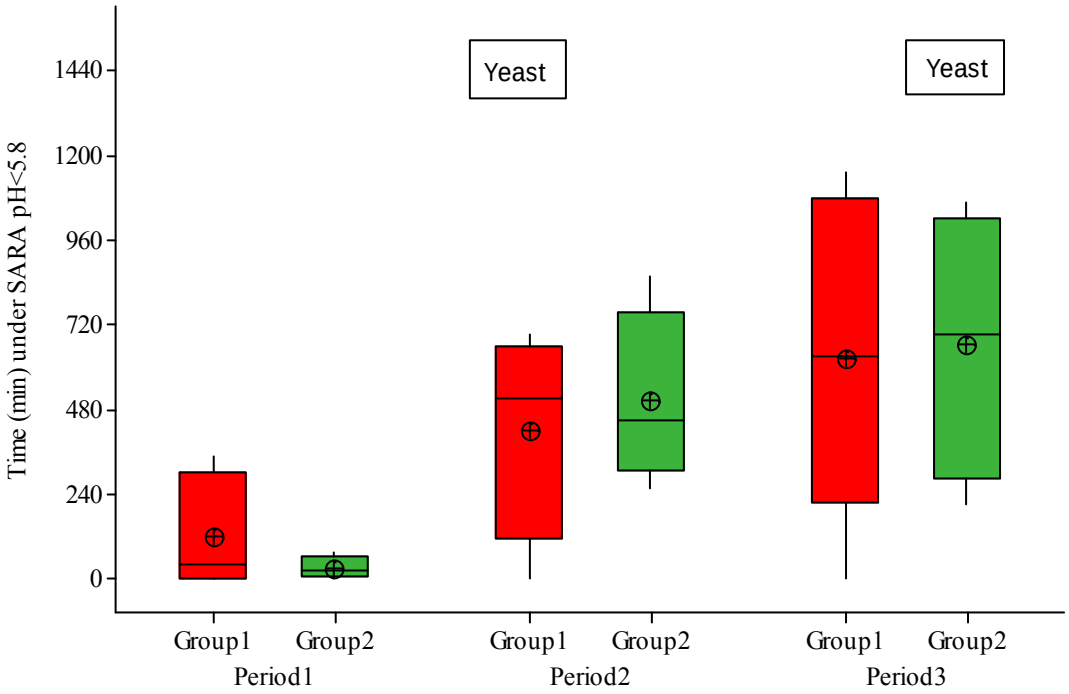

Figure 3.11 Time spent under SARA (pH<5.8) per Group and Period. Data is presented as interquartile range (box) and median (horizontal line within the interquartile range), with asterisk representing outliers, circle with central cross representing means and vertical line (whiskers) representing the lower and upper 25% of the distribution. Red boxes represent Group 1 while green boxes represent Group 2.

3.5.4 Discussion

The aim of Trial 2 was to evaluate the effect of yeast supplementation on cow performance, rumination time and rumen pH in cows fed an acidogenic diet. No statistically significant effects of yeast supplementation were observed on any of the parameters measured in the experimental Groups.

Despite the feeding regime, milk yield and milk characteristics are normally expected to present a declining trend after peak lactation (after six to eight weeks following calving) (Silvestre et al., 2009). Yeast supplementation was unable to counteract this trend as observed in Figure 3.7.

Previous research studies using artificially induced SARA did result in effects on performance parameters. Krause and Oetzel (2005) were able to induce SARA in mid to late lactation dairy cows by feeding 3.5 to 4.6 kg DM of a wheat-barley pellet, although no effect on DMI was observed. However the SARA challenge resulted in a statistically significant reduction of more than 2 kg of milk yield. These authors found no effect on other milk characteristics. Response to yeast supplementation in SARA induced animals reported in other research studies have been similar to our results. AlZahal et al. (2014) reported no effect of yeast supplementation on milk yield and characteristics in late lactating dairy cows under an acidotic regime (high grain diet 49:51 forage: concentrate DM ration achieved by replacing 20% of the TMR with wheat-barley pellets).

Rumination time was not affected by yeast supplementation, and no relationship with level of mean rumen pH or rumen pH dynamics (time spent under the SARA thresholds) was observed. Saliva produced during rumination is one of the elements involved in the regulation of rumen pH, and so it could be speculated that low rumen pH values would have a close relationship with rumination i.e. low rumen pH would result in low rumination activity. However this was not observed in Trial 2.

The acidotic challenge implemented in Trial 2 was able to produce SARA bouts for more than 5h a day in all the experimental animals. However there were considerable differences in how individual animals coped with the acidotic challenge in terms of

the level (how low) and extent (for how long (minutes per day)) of SARA observed in individual animals (Figure 3.9). Despite identical management regimes (identical in all aspects apart from their group housing pen), similar parity and lactation stage, individual animals show high variation in their susceptibility to suffer SARA. This could be explained by diet characteristics, differences in eating, ruminating and sorting behaviour, feed intake (which in turn will result in "animal specific" diet characteristics), saliva production level, unique microbial population, differences in the absorptive capacity of the ruminal epithelia, and rate of feed passage.

The thresholds used to assess SARA were 5.8 and 6.2 for more than 3 hours a day, which were achieved during P2 and P3 when yeast was supplemented (Table 3.9). These thresholds were longer in duration but not lower than those proposed by Gozho et al. (2007) (pH depression below 5.6 for more than 180 m / d). and also used by Li et al. (2012) and Khafipour et al. (2009). However the acidotic challenge utilised in this trial was sufficient to observe the levels of SARA expected.

Mean rumen pH decline and time spent under SARA increased throughout Trial 2 in both Groups (Table 3.9). Yeast supplementation had no effect on the rumen pH variables evaluated. On a study with non- lactating dairy cows, Chung et al. (2011) reported similar results. When evaluating two different yeast strains, the authors reported that one strain was no different from the Control Group, and the other strain reduced rumen pH and extended the duration of time spent under SARA thresholds. Contrasting results are presented by AlZahal et al. (2014). Using active yeast, and after induced SARA, the authors reported a lower time spent under the acidotic threshold pH < 5.6 (Treatment 122 ±57 min versus Control 321 ±53 min) for yeast supplemented cows.

In the present study, yeast supplementation had no effect on any of the variables measured, and no conclusive evidence on the benefits of yeast supplementation was found. Further studies with lactating cows at different lactation stages, or on different feeding regimes and different environments such as grazing are required.

Further research is also needed to explore the ability of individual animals to handle acidotic challenges. Penner et al. (2009) found that ruminal epithelial cells from what they called “resistant sheep” had a greater capability to absorb VFA. Similar experiments are required in dairy cows. Furthermore individual differences in rumen passage rates may contribute to differences in rumen pH.

Rumen pH is responsive to chewing behaviour (eating and ruminating), and rumen pH decreases following meals and increases during periods of rumination. Rumination stimulates salivary secretion which aids in rumen pH regulation by salivary buffer production (Allen, 1997). The relationship between rumination time and different levels and extent of episodes of SARA could be explored by means of using RC in trials with higher number of animals, in different stages of lactation and under different feeding regimes and acidotic challenges. The aim would be to determine animal-specific rumination patterns and their relationship with SARA.

Different levels and extent of episodes of SARA were observed in Trial 2, and it could be that a higher acidotic challenge is required (for example using a higher dose of supplemented wheat) to produce a more stable and widespread SARA response in all animals, sufficient to produce clinical signs such as changes in milk quality. Such higher degrees of SARA may produce a more challenging environment in which to determine the effects of any supplementation with yeast.

3.6 Trial 3

Yeast supplementation of commercial dairy cows under standard grazing conditions

3.6.1 Introduction

SARA is characterised by low rumen pH episodes of irregular duration and non-specific clinical signs. It is thought that SARA is a widely occurring digestive problem in dairy cows, not only under confined concentrate-based feeding systems but also in grazing systems. The dairy industry in the UK relies on animals grazing forage throughout the months of April to September. Changes in diet (e.g. from indoor PMR diets to outdoors grazing) can lead to changes in rumen environment which can lead to SARA. Furthermore pasture (ryegrass) contains a high concentration of rapidly fermentable carbohydrates with low fibre content which might result in high risk of SARA for grazing dairy cows (Bramley et al., 2008).

Studies on grazing animals experiencing SARA are scarce. However some research has been published from countries with systems predominantly pasture fed such as Ireland and Australia. The most cited research is that by O'Grady et al. (2008). Taking a cut-off point for affected animals to be rumen pH < 5.5 after 6h at grazing, the authors reported the prevalence of low rumen pH values in Irish grazing dairy farms to be more than 10% of the evaluated herds suffering from SARA. Similar results were reported by Bramley et al. (2008) from a survey carried out on 100 Australian herds, pasture based but with concentrate and PMR offered. The authors reported that 10% of the animals were acidotic with rumen pH <5.8 from samples collected via rumenocentesis 2 to 6 h after morning milking (concentrate feeding). These studies reported rumen pH values from samples collected via rumenocentesis, as it was the preferred sampling technique. However it has limitations as it is invasive and only provides a single time point measurement that does not reflect the dynamics or circadian rumen pH. Therefore the aim of Trial 3 was to evaluate the effect that yeast supplementation had on performance and circadian rumen pH in commercial dairy cows grazing grass.

3.6.2 Materials and methods

Trial 3 was conducted during May – Sep 2013. All procedures related to animals were approved by the Veterinary Ethical Review Committee (Reference: VERC 11-13) of the Royal (Dick) School of Veterinary Studies of the University of Edinburgh. The Trial had the same overall structure to that of Trial 1 and 2, and only specific details and differences are described.

3.6.2.1 Animals and Housing

Fourteen multiparous milking cows were selected and balanced for DIM (139 ± 4.5d) and parity (4 L). The cows were then randomly allocated to 2 different Groups: G1 (DIM =140 ± 6.3 d, L = 4) and G2 = (DIM 137 ± 6.8 d, L = 4), with 7 cows in each Group. All cows were pregnant at the beginning of the Trial.

3.6.2.2 Experimental Design

Cows were divided into two groups G1 and G2. The experiment was split into five Periods (P1, P2, P3, P4 and P5). P1 = baseline, P2 = baseline grazing, P3 and P4 = treatment and P5 = washout period. P1 and P2 lasted for three weeks, cows were given two weeks to adapt to the facilities and diets and measurements were recorded on the third week. P3, P4 and P5 lasted for four weeks: weeks one to three were for adaptation, and measurements were carried out on the fourth week. In P3 and P4, yeast supplementation at 4.0 g /cow / d (60 billion cfu / cow / d)) was carried out following a cross-over design (Table 3.10).

3.6.2.3 Diets, grazing and yeast supplementation

During P1, Cows were offered a partial mixed ration (PMR) consisting of: first cut grass silage 56.5% (fresh weight PMR proportion), wholecrop wheat silage 14.7%, dairy meal 19.9%,parlour concentrate 6.28% and molasses 2.62%). From P2 onwards cows were grazing a ryegrass (*Lolium perenne*) sward during the day and night. In

addition, when the cows came in for milking in the afternoon, they were offered a buffer PMR (first cut grass silage 45.5%, wholecrop wheat silage 35.4%, Langhill dairy meal 18.9%, and calcined magnesite 0.3%). Additional concentrate was fed to yield in the milking parlour (Table 3.11).

Yeast was supplemented following a cross-over design (Table 3.10) at a dose of 4.0 g / cow / d (50 billion cfu/cow/d) during P3 – P4. The yeast was top-dressed on the buffer PMR after fresh food was delivered at 0700 after the morning milking. Cows were locked in the feed trough for 15 min using the self-locking neck yoke mechanism to ensure the cows ate all the yeast supplemented. After this, the cows were moved outside with the rest of the herd to continue grazing.

3.6.2.4 Sampling procedures and measurements

Measurements were recorded on the last week of each experimental period (P1 and P2 = week 3 and for P3, P4 and P5 = week 4) as described in Section 3.3.4 and Table 3.1. Rumen pH was recorded using the same equipment (WellCow bolus) used in Trial 2 and as described in Section 3.5.2.4

Feed sampling and analysis

Every week samples (500 g approximately) of the forages used in the PMR were taken. During the periods when cows were outside grazing fresh grass, samples were collected each week from the paddock were cows were grazing using the hand-plucking technique (Pulido and Leaver, 2001). Samples were analysed at Bioparametrics Ltd. laboratory (Edinburgh, Scotland UK).

3.6.2.5 Statistical Analysis

Statistical analysis was performed as detailed in section 3.3.5. The model included fixed effects of period and treatment and the interaction of period and treatment with cow as the random effect.

Table 3.10. Timeline and experimental design used in Trial 3

	P1			P2			P3				P4				P5			
Week	1	2	3	1	2	3	1	2	3	4	1	2	3	4	1	2	3	4
Week	20th May	27th May	**3rd Jun**	10th Jun	17th Jun	**24th Jun**	1st Jul	8th Jul	15th Jul	**22nd Jul**	29th Jul	5th Aug	12th Aug	**19th Aug**	26th Aug	2nd Sep	9th Sep	**16th Sep**
Group 1	PMR + Concentrate			Grazing + PMR + Concentrate			**Grazing + PMR + Concentrate + 4 g yeast cow / d**				Grazing + PMR + Concentrate				Grazing + PMR + Concentrate			
Group 2	PMR + Concentrate			Grazing + PMR + Concentrate			Grazing + PMR + Concentrate				**Grazing + PMR + Concentrate + 4 g yeast cow / d**				Grazing + PMR + Concentrate			

3.6.3 Results

Table 3.11 shows the composition and chemical characteristics of the PMR fed on P1, and of the buffer ration fed from P3 onwards.

Table 3.11 Ingredients and chemical composition of the offered ration

Composition	Periods									
	Period 1		Period 2		Period 3		Period 4		Period 5	
Ingredient	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
Grass silage 1st cut	27	8.3	9	2.8	9	2.8	9	2.6	9	3.2
Wholecrop wheat	7	3.6	7	3.6	7	2.8	7	2.7	7	2.7
Langhill PMR meal	9.5	8.4	3.75	3.3	3.75	3.3	3.75	3.3	3.75	3.3
Molasses	1.25	0.9	0	0	0	0	0	0	0	0
Parlour Concentrate (fed to yield)	3	2.6	3	2.6	30	2.6	3	2.6	3	2.6
Calcined magnesite			0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Grazing (fresh grass)	0	0	45	10.5	45	21.2	45	13.8	45	9.1
Analysis										
DM (%)	49.9		31.1		46.6		34.7		28.3	
CP (% DM)	14.4		15.8		13.6		14.1		15.7	
NDF (% DM)	43		41		43		41		39	
uNDF forage (%DM)	11.8		12.7		14.4		13.2		11.7	
uNDF total (% DM)	17.7		14.8		15.8		15.1		14.0	
Oil (% DM)	4.4		3.4		4.1		3.4		3.3	
Sugar (% DM)	7.2		5.6		5.5		5.3		6.0	
Starch (% DM)	17.8		10.2		5.8		8.1		9.9	
Quick CHO (% DM)	15.9		17.1		17.4		18.2		18.4	
Slow CHO (% DM)	41.5		41.5		40.2		41		39.6	

Grazing quantities are estimates.

3.6.3.1 Milk yield and milk characteristics

Figure 3.12 shows the mean milk yield per Group for the entire duration of Trial 3, data is arranged by mean days in milk. Figure 3.13 shows the mean milk yield per Group obtained during the measurement week in each of the five experimental Periods.

No effect of yeast supplementation on mean milk yield was observed in any of the experimental Groups. An effect ($P<0.05$) of Period on milk yield was observed, with no interaction Period X Treatment observed (Table 3.12). No effect of yeast supplementation was observed on butterfat, milk protein or lactose content (Table 3.12)

3.6.3.2 Body condition score and body weight

Yeast supplementation had no effect on BW and BCS or change in body condition score across the experimental periods (Table 3.12).

3.6.3.3 Rumination time

Data on rumination activity was recorded and used to perform an evaluation of the RC on its ability to record rumination activity (Chapter 4). Due to the poor results obtained in grazing animals, the data was considered unreliable and therefore was not used for further analysis nor was reported in the present Trial 3 results section.

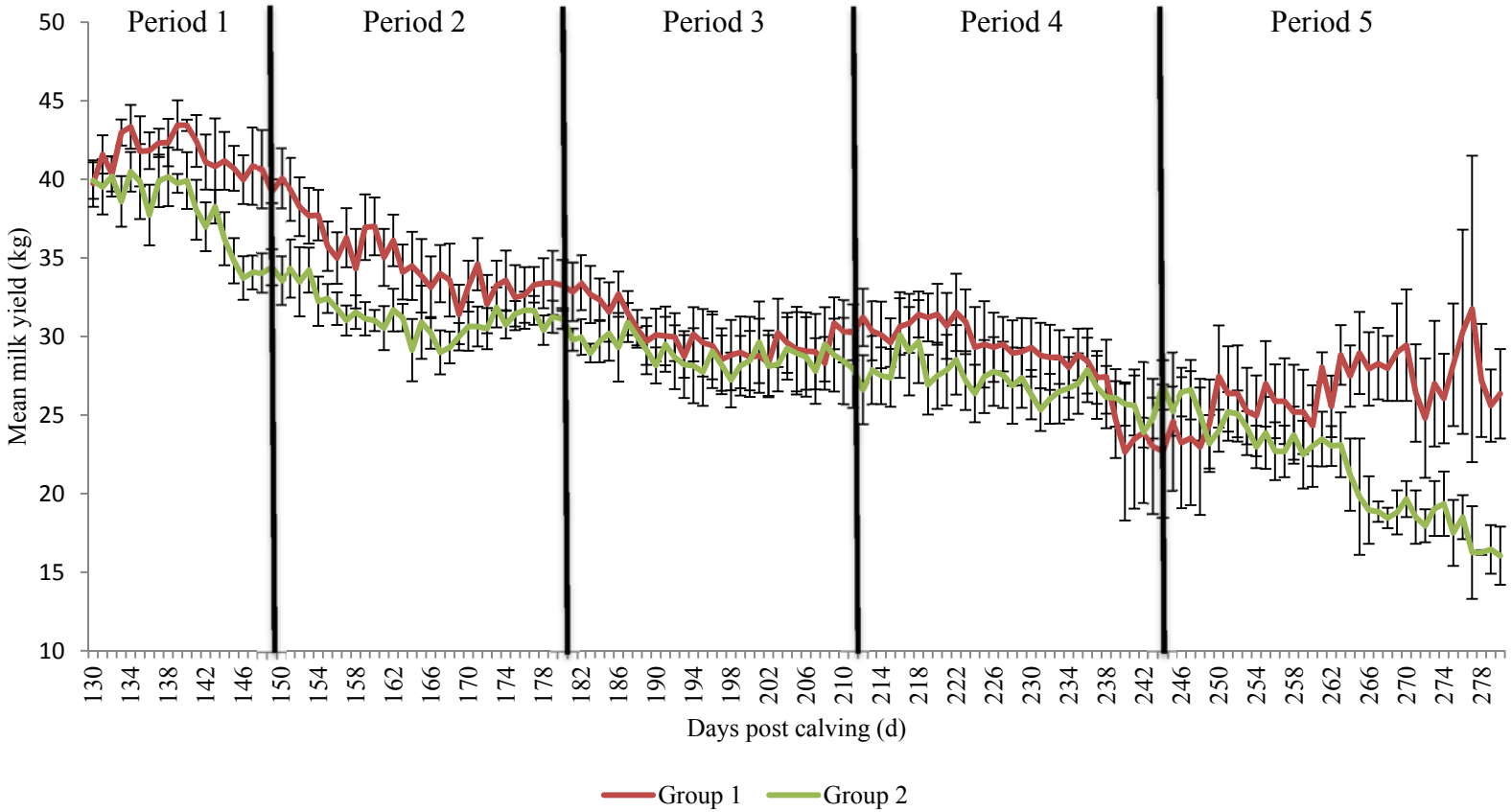

Figure 3.12 Group mean daily milk yield ((± SEM) by mean days in milk recorded throughout Trial 3. Group mean data was obtained from all seven experimental animals in each group. The vertical solid black lines represent the start and end of each experimental period.

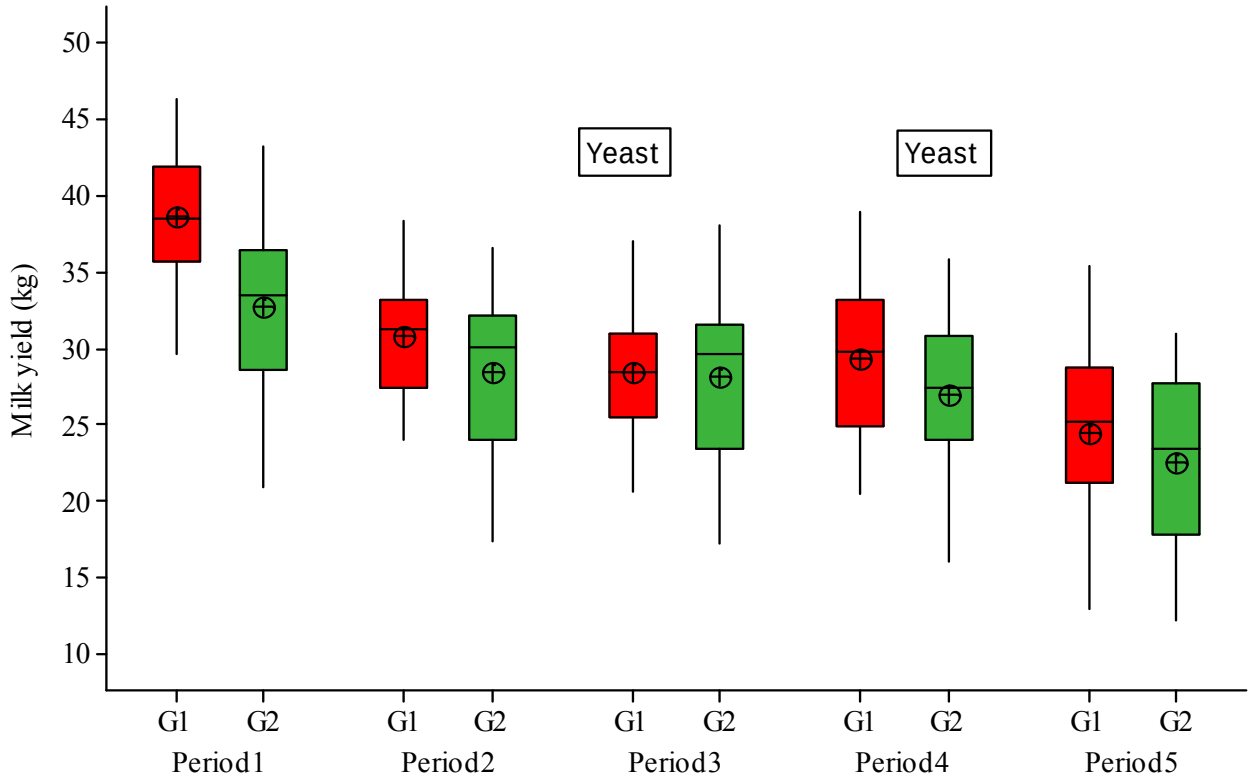

Figure 3.13 Group daily milk yield by Period. Data is presented as interquartile range (box) and median (horizontal line within the interquartile range), with asterisk representing outliers and circle with central cross representing means. Red boxes represent Group 1 and green boxes represent Group 2

Table 3.12 Effect of yeast supplementation on milk yield and characteristics throughout Trial 3

Variable	Group 1					Group 2					P value		
	P 1	P 2	P 3	P 4	P 5	P 1	P 2	P 3	P 4	P 5	Period	Tx	Inter
	Inside		**Yeast**			Inside			**Yeast**				
Milk yield (kg/d)	39 ± 1.77	31 ± 1.77	28 ± 1.77	29 ± 1.77	25 ± 1.77	33 ± 1.77	28 ± 1.77	28 ± 1.77	27 ± 1.77	23 ± 1.77	<0.001	0.31	<0.001
Butter fat (%)	3.94 ± 0.18	4.33 ± 0.18	3.97 ± 0.18	4.76 ± 0.18	4.59 ± 0.18	4.39 ± 0.18	4.65 ± 0.18	4.26 ± 0.18	4.75 ± 0.18	4.72 ± 0.18	<0.001	0.28	0.31
Protein (%)	3.00 ± 0.08	3.20 ± 0.08	3.08 ± 0.08	3.36 ± 0.08	3.48 ± 0.08	3.18 ± 0.08	3.23 ± 0.08	3.06 ± 0.08	3.36 ± 0.08	3.52 ± 0.08	<0.001	0.63	0.15
Lactose (%)	4.26 ± 0.09	4.42 ± 0.08	4.31 ± 0.08	4.34 ± 0.08	4.07 ± 0.08	4.45 ± 0.08	4.35 ± 0.08	4.30 ± 0.08	4.25 ± 0.08	4.09 ± 0.08	<0.001	0.93	0.23
BW (kg)	663 ± 17	651 ± 17	649 ± 17	644 ± 17	678 ± 17	664 ± 17	649 ± 17	669 ± 17	660 ± 17	703 ± 17	<0.001	0.62	0.16
BCS	2.75	2.50	2.25	2.50	2.25	2.25	2.50	2.50	2.25	2.50		0.40	
	P2 – P1	P3 – P2	P4 – P3	P5 – P4		P2 – P1	P3 – P2	P4 – P3	P5–P4				
BCS Change	-0.25	-0.25	0.25	0		0.25	-0.25	-0.25	0			0.62	

Mean ± SEM are presented for milk yield, milk characteristics and BW. BCS is presented as median. Tx = treatment, Inter = interaction

3.6.3.4 Rumen pH

Complete data on rumen pH values per hour were obtained from thirteen cows in P1 and P2, twelve cows in P3 and eleven cows in P4 and P5. No differences in rumen pH were observed as a response to yeast supplementation. A significant effect of Period was obtained (Table 3.13). When analysing the dynamics of rumen pH, different periods of time spent under the acidotic thresholds (time pH < 6.2 or 5.8) were observed. Cows tended to spend more time under the acidotic threshold as Trial 3 progressed (Figures 3.14 and 3.15). However no statistically significant effect of yeast supplementation was obtained. An effect of Period and an interaction Period X Treatment was obtained (Table 3.13).

Table 3.13 Effect of yeast supplementation on rumen pH and rumination time throughout Trial 3

Variable	Group 1					Group 2					P value		
	P 1	P 2	P 3	P 4	P 5	P 1	P 2	P 3	P 4	P 5	Period	Tx	Inter
	Inside		**Yeast**			Inside			**Yeast**				
Rumen pH													
Mean pH	6.34 ± 0.07	6.30 ± 0.07	6.22 ± 0.07	6.10 ± 0.07	6.14 ± 0.07	6.29 ± 0.07	6.23 ± 0.07	6.06 ± 0.06	5.85 ± 0.06	6.00 ± 0.06	<0.001	0.12	<0.05
Time pH<6.2 (min/d)	390 ± 126	490 ± 115	651 ± 117	847 ± 117	662 ± 121	548 ± 117	674 ± 105	993 ± 105	1324 ± 107	1005 ± 109	<0.001	<0.15	<0.05
Time pH<5.8 (min/d)	13 ± 104	16 ± 95	107 ± 97	301 ± 97	414 ± 100	53 ± 96	16 ± 87	219 ± 87	570 ± 88	468 ± 90	<0.001	0.38	<0.05

Mean ± SEM. Tx = treatment, Inter = interaction

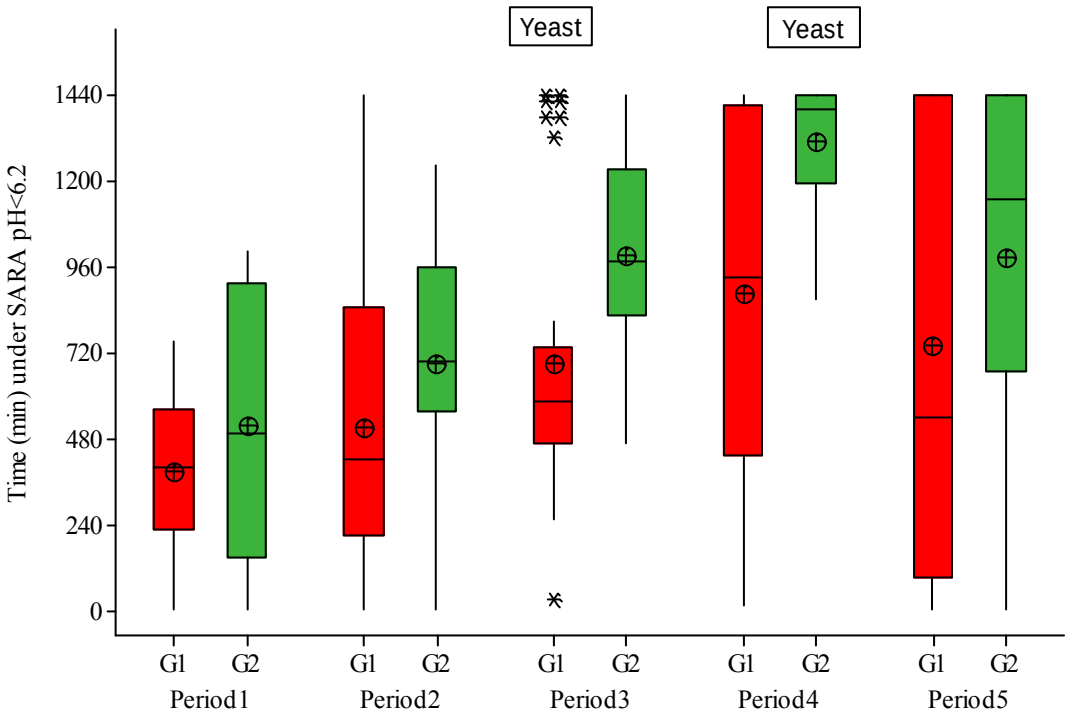

Figure 3.14 Time (min) spent under SARA (pH < 6.2) per Group and Period. Data is presented as interquartile range (box) and median (horizontal line within the interquartile range), with asterisk representing outliers and circle with central cross representing means. Red boxes represent Group 1 and green boxes represent Group 2.

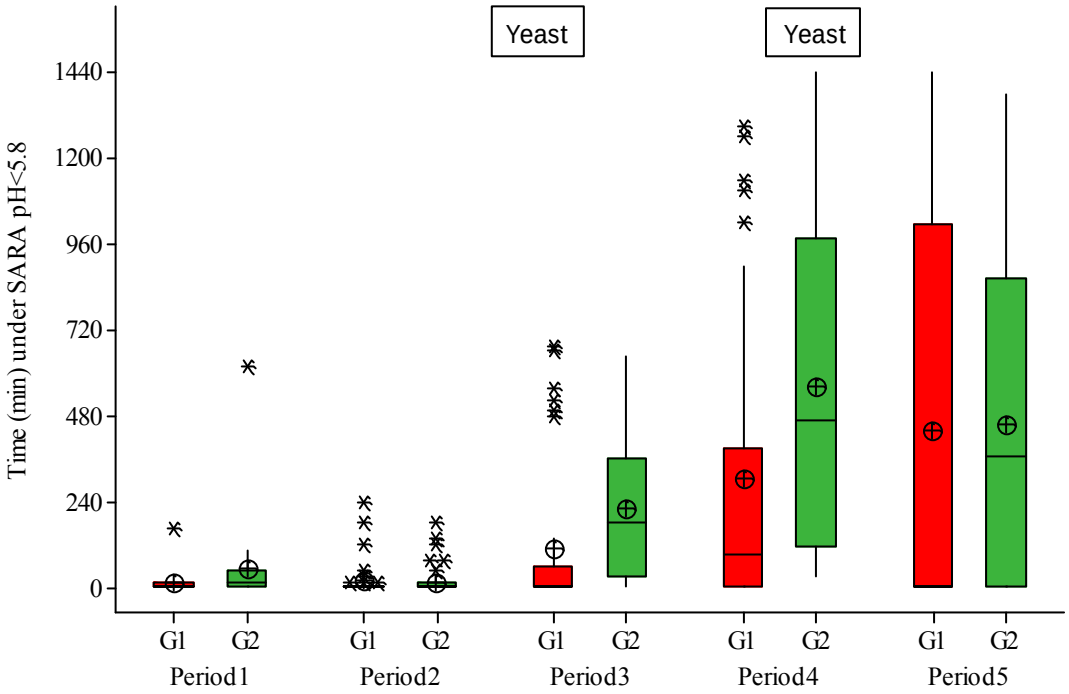

Figure 3.15 Time (min) spent under SARA (pH < 5.8) per Group and Period. Data is presented as interquartile range (box) and median (horizontal line within the interquartile range), with asterisk representing outliers and circle with central cross representing means. Red boxes represent Group 1 and green boxes represent Group 2.

3.6.4 Discussion

The aim of Trial 3 was to evaluate the effect of yeast supplementation on performance and rumen pH in cows grazing grass. No statistically significant effect of yeast supplementation was observed in any of the evaluated variables.

It is thought that cows consuming high quality pastures (for example ryegrass) might be at risk of suffering SARA (Kolver and de Veth, 2002; O'Grady et al., 2008). With this rationale, Irvine et al. (2011) evaluated the effect of yeast (fermentation product) on early lactation dairy cows consuming high-quality pasture. However the authors did not evaluate rumen pH as they considered that the cows would experience detrimental rumen pH levels. However no effect of yeast supplementation on performance and milk characteristics were found.

In Trial 3, milk yield declined throughout the experimental Periods following the "natural" lactation curve, with the most abrupt decline from P1 (indoors PMR) to P2 (outside grazing). Yeast supplementation was unable to counteract the decline in milk yield. Cows started the Trial averaging 139 DIM when lactation peak has passed, and milk yield is normally declining. Furthermore it is thought that grazing conditions are not capable of achieving the same milk yields compared to PMR based diets in high yielding cows. This is because grazing animals are unable to meet the nutritional requirements for high levels of milk production, as dry matter intake is low and the diet is of lower quality (pasture compared to PMR) which results in lower energy intakes (Stockdale, 1999).

Previous research has suggested that changing from indoor PMR feeding regimes to outside grazing poses a risk for the incidence of SARA (O'Grady et al., 2008). In the current study, a tendency for the decline in mean rumen pH and increase in the time spend under SARA threshold (pH <6.2) was observed when cows moved from indoors to outside grazing. Similar results were reported by Al Ibrahim et al. (2012), where the authors reported differences in rumen pH level when cows were subject to two different management strategies: abrupt or gradual introduction to pasture. The abrupt introduction to pasture produced the lowest levels of mean rumen pH and time spent under SARA (pH<5.8). Similar results were reported by Gasteiner et al. (2015)

when measuring rumen pH in cows under a gradual transition from PMR to grazing. The authors found a decrease in mean rumen pH when the cows were first introduced to pasture (2 – 7 h grazing), but an increase in mean rumen pH when grazing time was gradually increased (Gasteiner et al., 2015). The results obtained in Trial 3 for time under the SARA thresholds and mean rumen pH differed from those of Gasteiner et al. (2015), as in Trial 3 rumen pH continued to decline throughout the periods until P5 when a slight increment was observed. These differences could be explained by the duration of the experimental periods, which were 7d in the reported study compared to 28 d in Trial 3. Furthermore a higher level of milk yield reported in Trial 3 would require higher levels of grass intake, which may have stronger effects on rumen pH.

Although a decline in mean rumen pH can be observed across the experimental periods, the decline tended to reduce by P4 and P5 (Table 3.13). This is corroborated by the wide spread of values observed in the time that cows spent under the SARA thresholds in P4 and P5 (Figures 3.14 and 3.15). It could be hypothesised that for some cows, an adaptation to grazing conditions could improve rumen environment hence maintaining rumen pH levels. However other cows consistently produced low rumen pH values throughout Trial 3, suggesting that there are individual cow factors influencing rumen pH.

Furthermore the results obtained for both time under SARA and mean rumen pH suggested that in Trial 3, the effect or challenge of grazing on rumen pH was not considerable. This could be due to firstly the quality of the offered pasture, secondly the high variation in individual feed intakes that is thought to occur with grazing animals. This could explain the wider variation in rumen pH recorded compared with indoor trials. Lastly the effect that the buffer feed diet has on grazing, where it is thought that buffer diets provide effective fibre capable of counteracting the low levels of fibre and high non-structural carbohydrates present in pasture. Furthermore it is thought that buffer diets may affect grazing feeding behaviour, which may reduce the levels of SARA challenge in the cows.

Yeast supplementation had no effect on rumen pH or time spent under the SARA thresholds in the current study. Similar results were obtained by Al Ibrahim et al. (2012) in early lactation fistulated cows. However the authors reported an effect of yeast supplementation on the time that animals spent under SARA conditions.

In Trial 3, yeast supplementation had no effect on any of the variables under study in cows under standard grazing conditions. This could be due to the moderate or low levels of SARA observed, which in turn could be explained by the time given to the cows to adapt to the change in diets (PMR indoor feeding versus grazing) and the effect of the buffer feed offered to the animals. Furthermore analysis of the type and quality of sward grazed by the cows showed it to be a ryegrass sward in a late stage of maturity containing medium levels of sugar (40% NDF as % of DM, and sugar content of 9 % DM), which will have reduced the potential acidotic challenge.

Additional studies with lactating cows at different stages of lactation (e.g. transition period and early lactation) are advisable to further explore the occurrence of SARA under grazing conditions. Furthermore different grazing environments need to be evaluated. It is thought that the highest occurrence of SARA is observed when cows are exposed to grazing young swards with high nutritive characteristics and low effective fibre.

Measuring intake and rumination activity is challenging in grazing environments, and is not possible with current technology (see evaluation of rumination collars in Chapter 4). However, as mentioned previously, chewing activity can strongly influence rumen pH. Therefore further research is required to explore the relationship of feed intake and rumination time in the development of SARA under grazing conditions.

3.7 Discussion

It is thought that SARA has detrimental effects on dairy cow health and productivity. On the one hand, rumen pH lower than 5.6 for more than 3 h a day could affect feed intake, milk production and characteristics, and cause diarrhoea and lameness. However these effects are not always present (Plaizier et al., 2008). On the other hand, mean rumen pH lower than 6.2 can have detrimental effects on ruminal cellulolytic bacteria and fibre digestibility (Mould et al., 1983), which may not be obvious clinically in the cow.

Several nutritional interventions have been devised to counteract SARA, including yeast supplementation. It is hypothesised that yeast stabilises rumen environment and hence increases rumen pH. However the effects of yeast supplementation on rumen pH are contentious and contrasting results are reported in the literature with no effects, mild responses or clear positive effects on performance and rumen pH. Such results were backed-up in this study.

The aim of the Trials was to evaluate the effect of yeast supplementation on performance, rumination time and rumen pH in cows fed a standard commercial diet, an acidogenic diet and when at grass grazing.

No statistically significant effects of yeast supplementation were observed on any of the parameters measured in any of the three Trials carried out. These results are in line with previous studies that found marginal or non-significant differences for production performance, rumination time or rumen pH variables.

The lack of effect of yeast supplementation could be due to the low prevalence of SARA in Trials 1 and 3, and a moderate acidotic challenge in Trial 2. It is thought that yeast supplementation has positive effects when cows are under physiological or nutritional stress, such as in early lactation or when higher levels of inclusion of concentrate or high levels of starch content. These challenges were not observed in Trials 1 and 3, as can be observed by mean rumen pH levels higher than 6 for both Trials in almost all of the cows.

Although in Trial 2, the acidotic challenge was able to reduce rumen pH (mean pH below 6.0 during the experimental periods), the level and extent of SARA was barely similar to those reported by Gozho et al. (2005) of rumen pH< 5.6 for more than 170 min /d, or pH <5.6 for more than 180 min /d reported by AlZahal et al. (2007a). Therefore the degree of SARA may not have been sufficient to see any effect. It is possible that an effect of yeast supplementation could be observed in more rigorous acidotic challenges with different levels (rumen pH < 5.8) or extent (longer periods of time) of SARA.

A study using larger number of animals could provide more data to account for individual animal variation, and may enable differences in performance due to treatments to be more easily detected. In these Trials, infrastructure and other resources determined the number of experimental animals. Bruno et al. (2009a) using more than 700 animals found an effect of yeast supplementation on performance on multiparous cows. Similar results were observed by Shaver and Garrett (1995) when looking at the effect of yeast supplementation on dairy cows in 11 different farms accounting for more than 100 animals.

Most of the research carried out on the effect of yeast supplementation has been on high concentrate, low fibre diets. To our knowledge, there are no studies that report yeast supplementation using standard commercial diets without any confounding factors associated, such as acidotic or environmental challenges. Most research (especially in America) relies on maize silage, and these feeding regimes based on maize silage contain higher levels of starch content. Starch increases lactic acid production that can lower rumen pH and reduce fibre digestion, creating more acidotic challenge. This current study using grass silage is unique in our experience when looking at the potential effect of yeast supplementation.

Moreover it is seldom that work has been conducted on pasture based systems, even though it is thought that high quality forage (e.g. ryegrass, lucerne) may affect rumen pH levels. From the results of Trial 3, we could hypothesise that rumen pH and therefore cow performance and health may not be as compromised by grazing forage as previously suggested. The observed levels of rumen pH and SARA were very

similar in Trial 3 to those observed in housed cows consuming mixed rations in Trial 1. These results potentially add to the debate over the prevalence and significance of SARA in grazing animals, and suggest that SARA in grazing animals may not be as prevalent as some studies using rumenocentesis suggest.

Previous studies on rumen pH have relied on rumenocentesis or other more invasive techniques, which do not provide information on circadian pH fluctuation. These techniques are a constraint to the number of animals evaluated, with very few studies reporting more than 5 animals, and even fewer are able to report circadian pH for longer than a couple of days. This could mask the effect of yeast supplementation and any other supplement on rumen pH, as it might be that the actual period of action could be short, missed by the rumen sampling technique used or even overestimated. Furthermore the use of one or few sampling points during the day may mask the highly varied animal response observed. Huge individual variations were observed in all Trials regarding the level and duration of SARA between animals, showing a different level of individual cows to cope with the acidotic challenge in Trial 2.

The use of rumen boluses and similar devices represents the best option for evaluation of the effects of yeast supplementation on rumen pH. These devices are advantageous as their capabilities for continuous measurement of rumen pH provide a “dynamic” portrait of circadian rumen pH, and allow the measurement of extent and duration of SARA during days or even weeks. Current cost, lifespan and interpretation of the large amount of data produced currently limit their application commercially.

Yeast supplementation had no effect on rumination time, and these results are in line with those reported by Devries and Chevaux (2014) which only found a tendency for rumination time to be longer in dairy cows supplemented with yeast. Similar results were obtained with dairy goats, where yeast supplementation had no effect on rumination time (Desnoyers et al., 2009b).

The results obtained in this Chapter are in line with literature that report no effect of yeast supplementation on performance, rumen pH and rumination time. It is

noteworthy that although no statistically significant differences between milk yields were observed, an arithmetical difference of more than 1 kg milk between Groups were observed, with a marginal response obtained in yeast supplemented cows. Further research is needed in the use of yeast under differing dietary and animal conditions to examine the cost:benefit of yeast supplementation in commercial dairy herds.

Chapter 4 Results

Assessment of rumination in cubicle housed and grazing dairy cows

Adapted from: Ambriz-Vilchis V., Jessop N.S, Fawcett R.H., Shaw D.J., and A.I. Macrae. (2015) Comparison of rumination activity measured using rumination collars against direct visual observations and analysis of video recordings of dairy cows in commercial farm environments. Journal of Dairy Science Vol: 98, Issue: 3 Pages 1750 – 1758 (available in Appendix 1).

4.1 Introduction

Changes in rumination time could be used as a proxy measure of illness or changes in health status i.e. if detected, subtle changes in rumination activity could help in the detection of subclinical diseases before they progress and become a clinically apparent concern. To further investigate this possibility, accurate and precise methods to measure rumination time are required.

Traditionally visual observation is used to measure rumination, either through direct observation or by analysis of video recordings. Direct observation is the standard and more reliable method, however it presents some disadvantages e.g. requires trained personnel, and the number of animals that can be observed at one time is limited. Analysis of video recordings on the other hand allows observation of groups of animals, and can be performed outwith the study site. It is nonetheless limited as it requires trained personnel and relies on expensive infrastructure. Both methods are labour intensive, requiring prolonged periods of observation in order to obtain reliable information.

To overcome the difficulties posed by monitoring and recording behaviour, automated equipment to record feeding behaviour (eating and/or ruminating) have been developed. Automatic recording systems have the advantage of recording behaviour that could be missed by human observers. In the long term, the use of such

monitoring equipment could be more cost effective than human labour as the devices can be used to monitor several animals at the same time and in different productive settings. However these devices may be uncomfortable for the animal and could affect their normal behaviour. Also it is necessary for the equipment to be tested and validated to ensure that the obtained data is reliable and accurate.

In the past decades, various devices have been developed. These devices can measure rumination by means of analysing jaw movements (Beauchemin et al., 1989; Braun et al., 2013; Kononoff et al., 2002; Rutter et al., 1997; Umemura et al., 2009) or recording sounds of mastication (Bar and Solomon, 2010; Clapham et al., 2011; Elischer et al., 2013; Goldhawk et al., 2013; Laca and WallisDeVries, 2000; Navon et al., 2013; Schirmann et al., 2009). Some of these devices have been validated in different experimental conditions and with variable results (Table 1.2)

Although the performance or output of the RC has been under scrutiny in the past years, the consensus seems to be that further validation is needed (Burfeind et al., 2011; Elischer et al., 2013; Goldhawk et al., 2013; Schirmann et al., 2009). Therefore the aim of the present study was to compare rumination activity measured with the RC against that obtained from direct observation and by analysis of video recordings in commercial settings with both cubicle-housed and grazing dairy cows.

4.2 Materials and Methods

Three Trials (Trials 1, 2 and 3) were conducted at the University of Edinburgh at Langhill Farm, Roslin (Midlothian, Scotland, UK) during 2012 and 2013. The Trials are described in detail in Chapter 3. A brief outline is given as follows.

4.2.1 Animals and housing

In each Trial fourteen multiparous milking cows (unique to each Trial) were selected and balanced for DIM and parity. The cows were then randomly allocated to two different groups of seven cows to facilitate management routines e.g. milking, and in Trial 1 so they could be easily video recorded. All the individuals were clearly identified with a unique number or letter by colour spray (Arco Ltd., England UK). Cows were offered a PMR with additional concentrate fed to yield in the milking parlour.

In Trials 1 and 2, all cows were housed in cubicle shed. In Trial 3 cows were grazing a ryegrass (*Lolium perenne*) sward during the day and night, and cows were also offered a buffer PMR ration for two hours after the afternoon milking. In all Trials water was supplied *ad libitum*, and the cows were milked twice a day at 0500 and 1500 h as per standard farm practices. Cows were given 2 weeks to adapt to the diet, facilities and the RC. All measurements were taken in the third week.

4.2.2 Data collection

In all trials, a RC (Qwes-HR Lely Ltd., St. Neots, UK) was fitted to each cow to record rumination. The raw data from the RC were collated and the output presented as rumination in min per 2-h periods (e.g. 0200 h, 0400 h, 0600 h, 0800 h or 0100 h, 0300 h, 0500 h, 0700 h, and so on) over a day.

4. 2. 2. 1 Video recording of cow behaviour (Trial 1)

Cow behaviour was recorded using 16 video cameras. The cameras were positioned in key places throughout the shed (fitted to the roof 4.0 and 5.5 m above the ground) so that all cows were viewed and easily identified (by their unique number or letter) at any given time. The area under observation was naturally lit during daylight hours and infrared lighting was used for night time recording. The cameras recorded 24 h / d. On an average day, 3 h of cow behaviour was missed as the cows left the pens to be milked (around 0500 and 1500 h). Behavioural measurements were analysed and

recorded using The Observer software (Noldus Information Technology, 2004, Wageningen, the Netherlands) by one trained observer using the videotapes recorded during the measuring week. Each cow's behaviour was recorded continuously for periods of 2 h at a time to complete a full 24-h period per week.

4.2.2.2 Direct observation of cow behaviour (Trials 1, 2, 3)

Cow behaviour was recorded by one trained observer using a handheld device (Psion WorkAbout Pro M, Noldus Information Technology).

Behaviours (eating, drinking, idling, and ruminating) were recorded according to the ethogram shown in Table 2.1 Chapter 2. Rumination was defined as the time a cow spends chewing a regurgitated bolus until it swallows it back, either standing or laying down. Behaviours were recorded continuously (Martin et al., 1994; Mitlohner et al., 2001) and were defined as being mutually exclusive categories. The 2-h periods recorded were selected so that they matched exactly the period reported by the RC; behaviours were reported in min per 2 h. Direct observations were recorded to match exactly the periods reported by the RC and were selected to match 2h periods recorded with the RC.

Each cow was recorded continuously for periods of 2 h without interfering with their normal behaviour: a) when cows were housed indoors (Trials 1 and 2), the observer was standing in places of the shed where all the behaviours of a specific animal were easily recorded and the observer's presence had no effect on the cow's routine and behaviours (i.e., the animal did not change behaviour or move away from observer); b) when cows were outside grazing on pasture (Trial 3), the observer was standing in the field at a distance (approximately 10 m) where all the behaviours of a specific animal were easily recorded and the observer's presence had no effect on the cow's routine and behaviour (i.e. the animal did not change behaviour or move away from the observer).

Data was collected from periods where no treatment was given i.e. Trial 1 Period 1, Trial 2 Period 1 and Trial 3 Period 2 (Tables 3.2, 3.6 and 3.10 in Chapter 3).

4.2.3 Statistical Analyses

4.2.3.1 Observer Reliability

To test the observer reliability when assessing behaviours from the video recordings, the trained observer scored behaviours (rumination time, eating, drinking and idling) twice on 20% of the total observed 2-h periods and the Pearson correlation coefficient between the measurements was calculated.

4.2.3.2 Rumination collars versus video recordings

Trial 1: To evaluate the relationship between the rumination times obtained with RC and analysis of video recordings, a modification of the standard limits of agreement (LoA) methodology was adopted to take account of the multiple observations per individual cow (Bland and Altman, 2007; Bland and Altman, 1986). When considering the relationship between the 2 variables, a standard linear mixed effect model was used to resolve the non-independence associated with the multiple measurements per cow (Paterson and Lello, 2003). In the linear mixed effect model, which cow that the measurement had come from was entered as the random effect. Additionally, an analysis was made to test whether the slope between RC and analysis of video recordings was different from 1.

4.2.3.3 Rumination collars versus direct observations

Trial 1: only one measurement was recorded for each individual cow. Therefore to evaluate the relationship between rumination times obtained with the RC and direct observations, a standard regression analysis and the standard LoA methodology were used.

Trials 2 and 3: The standard linear mixed-effect model (described above) and the modified LoA method with multiple observations per individual were again used.

Additionally, an analysis was made to test whether the slope between RC and direct observations were different from 1.

All statistical analyses were carried out using R (R Core Team, 2013) with the linear mixed-effect analysis carried out using the nlme package (version 3.1-113), the standard LoA method using MethComp package (version 1.22) and a modified version of the LoA with repeated measures as modified by Nutter (2008). Statistical significance was taken as $P < 0.05$.

4.3 Results

4.3.1 Observer reliability

Thirty three 2-h periods [20% of the total 2-h observed periods (196)] were analysed twice. The twice observed 2-h periods reported very similar rumination (Figure 4.1), drinking (Figure 4.2), eating (Figure 4.3) and idling (Figure 4.4) times, with a very strong positive correlation between the behaviour times obtained from the twice analysed periods (Table 4.1).

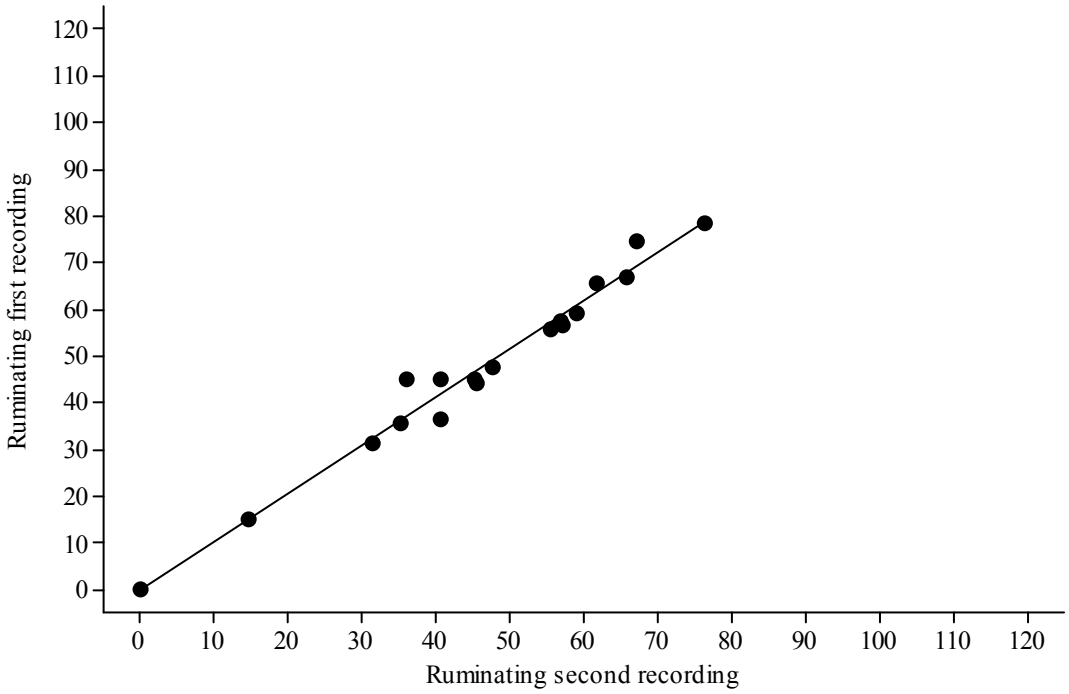

Figure 4.1 Relationship between ruminating time (min/2h) measured twice by the trained observer from the same 2 h periods Trial 1.

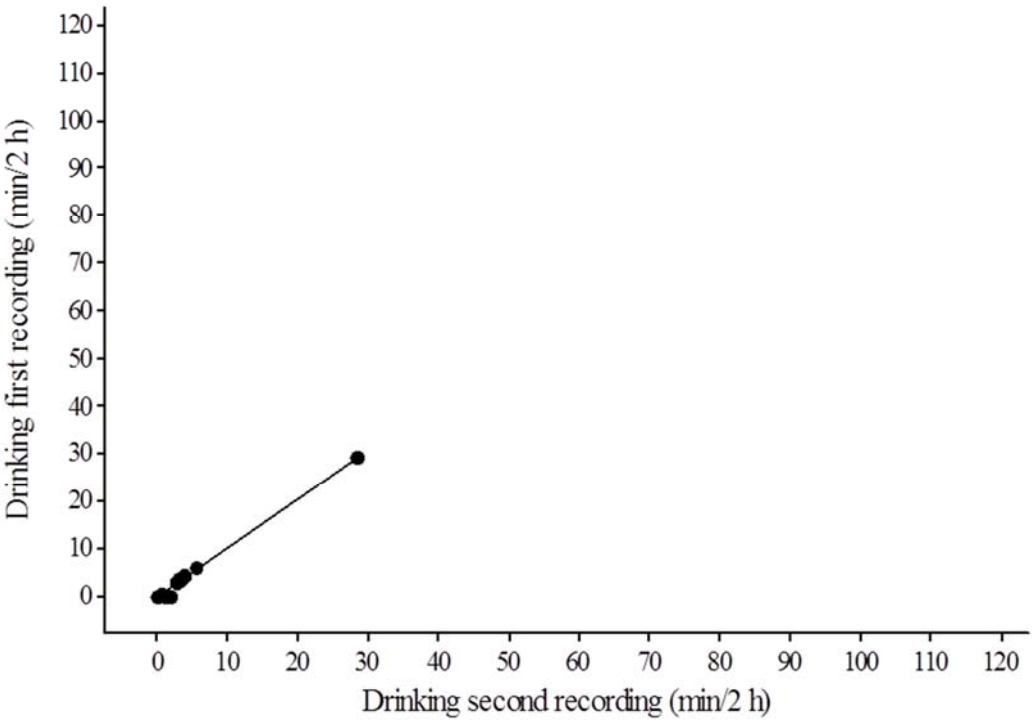

Figure 4.2 Relationship between drinking time (min/2h) measured twice by the trained observer from the same 2 h periods Trial 1.

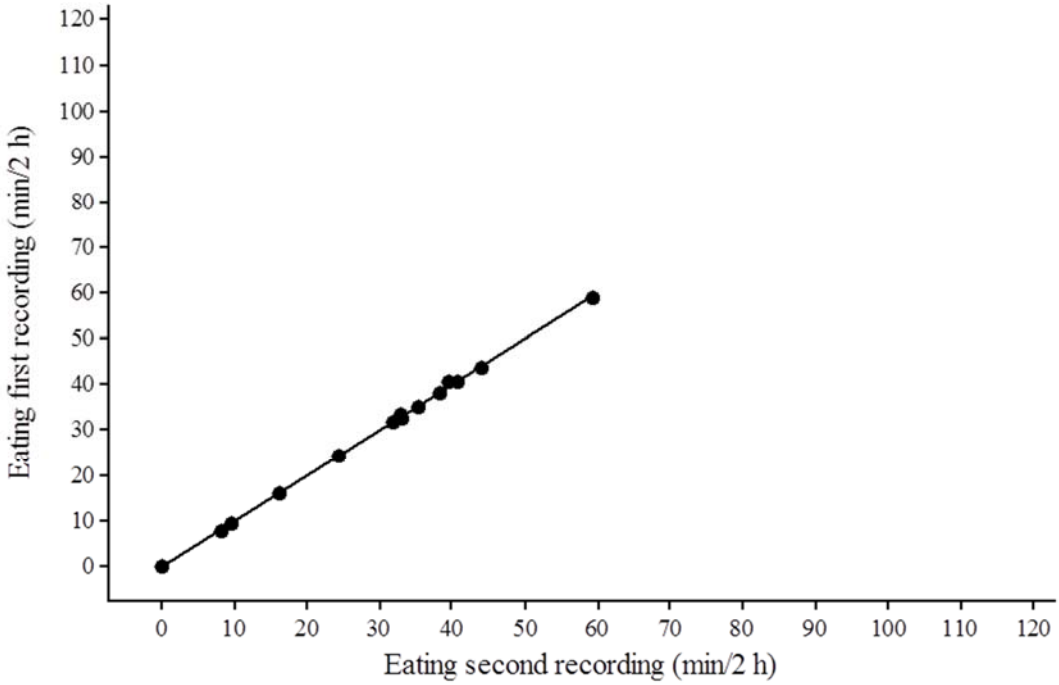

Figure 4.3 Relationship between eating time (min/2h) measured twice by the trained observer from the same 2 h periods Trial 1.

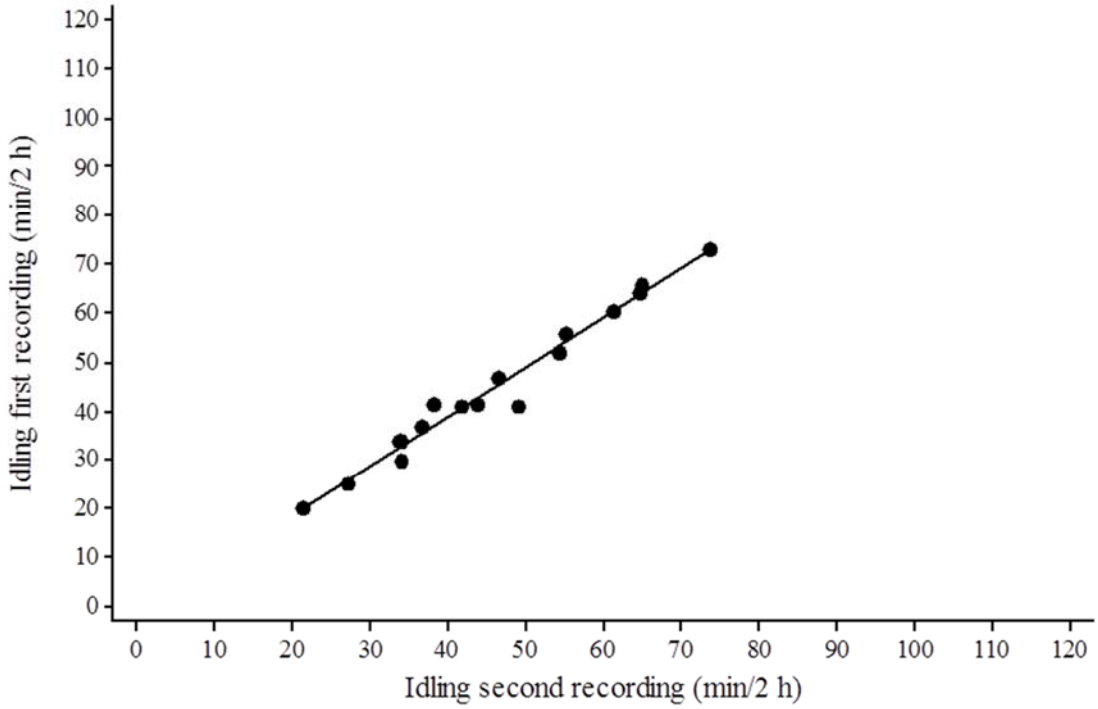

Figure 4.4 Relationship between idling time (min/2h) measured twice by the trained observer from the same 2 h periods Trial 1.

Table 4.1 Analysis of the relationship between the behaviours measured twice by the trained observer

	First recording mean ± sem	Second recording mean ± sem	r	P value
Rumination	39 ± 4	38 ± 4	0.99	< 0.001
Eating	19 ± 4	17 ± 4	0.94	< 0.001
Drinking	5 ± 3	5 ± 3	0.99	< 0.001
Idling	31 ± 5	34 ± 5	0.91	< 0.001

4.3.2 Rumination collars versus video recordings

In Trial 1, behaviour was recorded in 164 two hour periods from all cows. However only 136 two hour periods when cows were visible at all times were used for the analysis to determine the relationship between rumination time recorded by the RC and that obtained from analysis of video recordings. The RC recorded a mean rumination time of 45 ± 2 min/2h that was similar to the mean rumination time obtained by analysis of video recordings 46 ± 2 min/2 h (Table 4.2). The LoA plot (Figure 4.5) shows an evenly distributed scatter of measurements with no patterns, and there was no clear tendency for the difference between methods to become either larger or smaller as the averages increase. The RC reported rumination times that were on average 1 min (95% CI – 24 and 27 min) shorter than those recorded by analysis of videos.

Table 4.2 Analysis of the relationship between rumination times (min / 2 h) obtained with rumination collar (RC) and analysis of video recordings and direct observations: regression analysis (Trial 1 direct observations vs RC), Limits of Agreement method (all Trials) and mixed effect model (Trial 1, Trial 2 and 3 direct observations vs RC)

Trial		Regression Analysis lm(Obs~RC)					Limits of Agreement method			Mixed effect model lme(Obs~RC,~1|cowid)		
		N	R^2	Regression Equation	Std.Err	P value	Lower limit	Mean	Upper limit		Std.Err.	P value
1	Video vs RC	136					-26.92	-1.32	24.27	Video=0.53 + 1.02RC	0.051	< 0.001
1	Direct vs RC	14	0.66	Direct = 0.71 + 1.08RC	0.213	<0.001	-28.54	-3.29	21.98	-	-	
2	Direct vs RC	28					-32.56	-6.36	19.84	Direct=8.24 + 0.93RC	0.136	< 0.001
3	Direct vs RC	28					-51.16	0.93	53.02	Direct=17.66 + 0.57RC	0.207	< 0.05

lm= linear model, lme= linear mixed effects model.

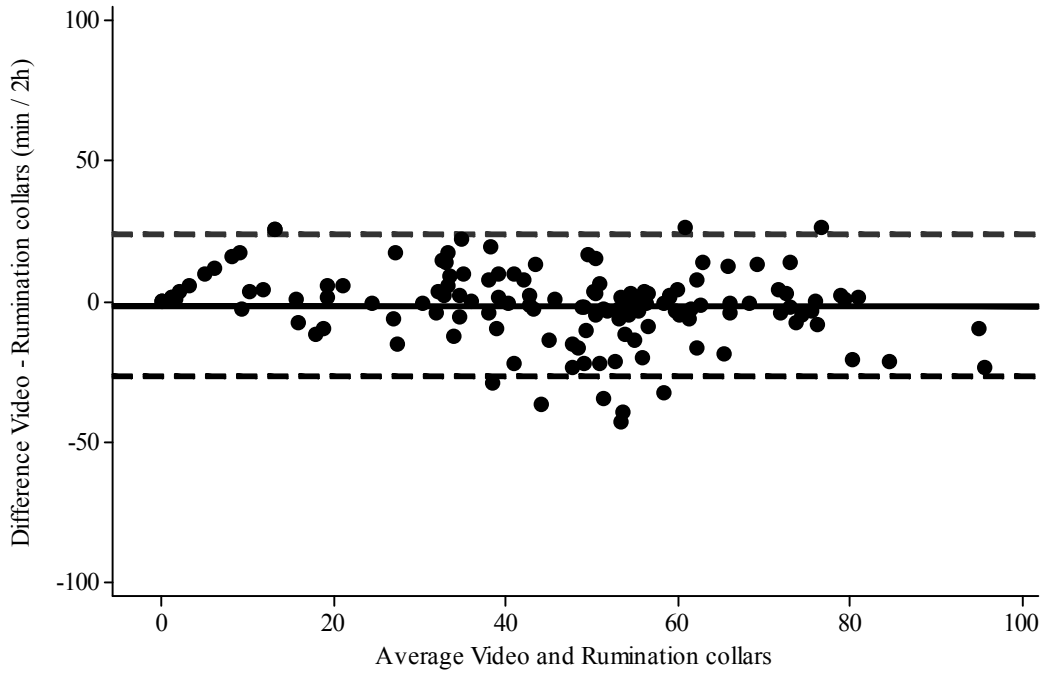

Figure 4.5 The Limits of Agreement method with multiple observations per individual, the plot shows rumination time (min/2 h) obtained with the Rumination Collars and analysis of video recordings in Trial 1. A total of 136 2 h periods were recorded from 14 separate cows. The lines represent the mean difference between the two methods (central horizontal solid line – 1 min) and the limits of agreement (broken lines) higher (upper horizontal line 25 min) and lower (lower horizontal line -27 min).

Individual plots of the relationships between the 2 methods showed large variation in the rumination time recorded (R^2 varying from 28.3 to 97.6% with slopes from 0.74 to 1.43, Figure 4.6). The variability per individual is best exemplified by cows Cd and T1, with poor agreement for cow Cd whereas data points that match almost entirely with the line of perfect agreement for cow T1.

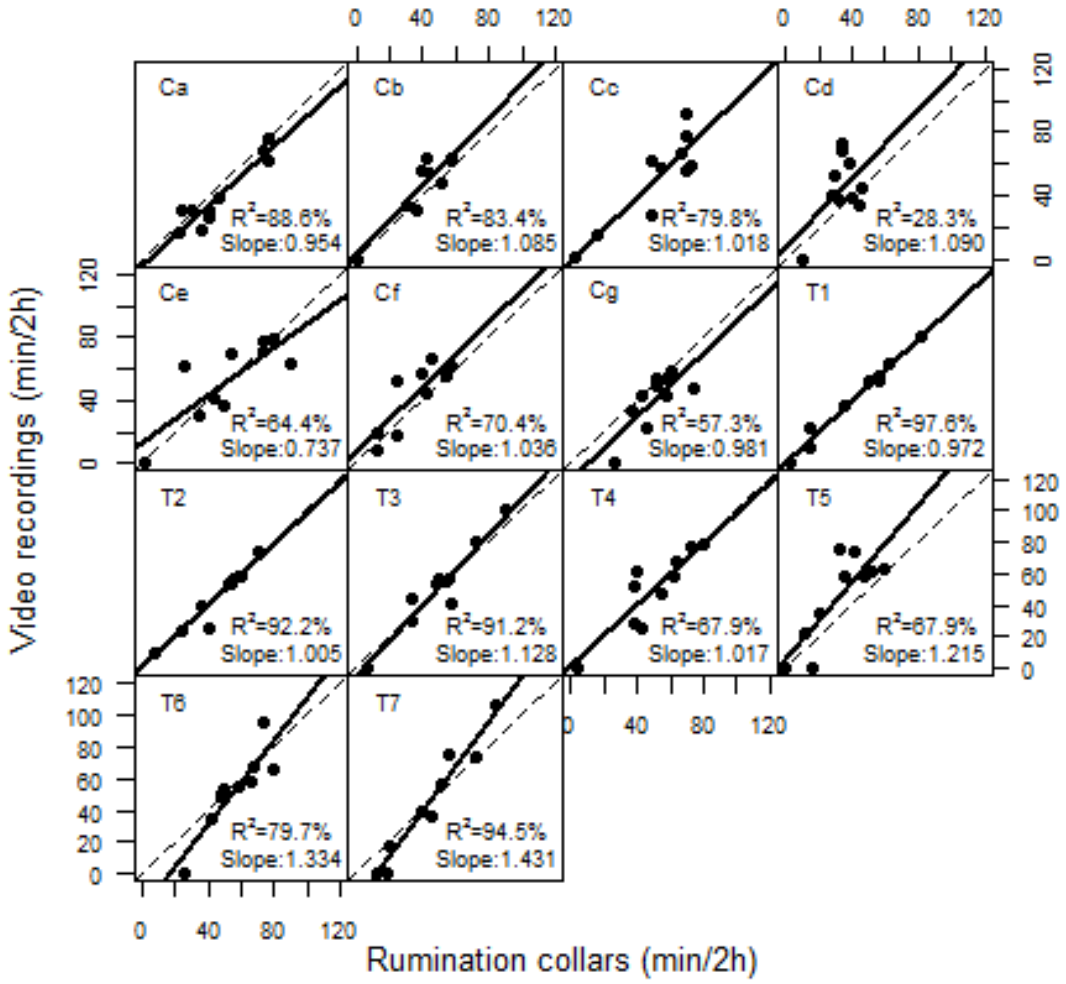

Figure 4.6 Relationship between rumination time (min / 2 h) measured by rumination collars and analysis of video recordings in Trial 1. Each panel represents data from one individual cow. The broken line depicts the line of equality on which all points would lie if RC and analysis of video recordings gave exactly the same results. The solid line shows the equation line.

If the data from all cows were considered, then a significant positive relationship was observed (P = 0.001, Figure 4.7), with the slope very close to 1 (slope = 1.02, Table 4.2). Excluding cow Cd from the analysis made little difference to this (Slope = 1.02). In either case, the slope was not different from 1 (P = 0.72).

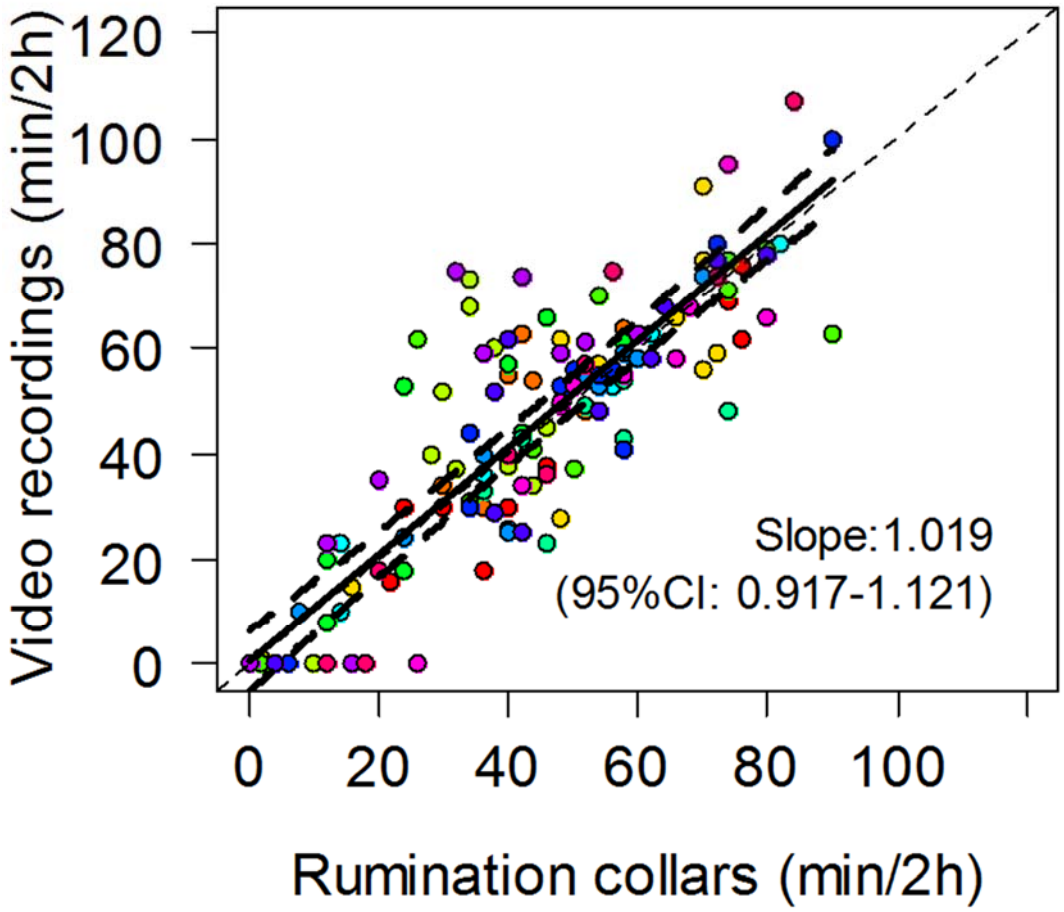

Figure 4.7 Relationship between rumination time (min / 2 h) measured by rumination collars and analysis of video recordings in Trial 1. A total of 136 2 h periods were recorded from 14 cows. The broken line depicts the line of equality on which all points would lie if RC and analysis of video recordings gave exactly the same reading every time. The solid line shows the equation line and the broken thicker lines show the 95 % confidence interval. Dots of same colour represent recordings made from the same cow.

4.3.3 Rumination collars versus direct observations

In Trial 1, behaviour was recorded in fourteen 2-h periods (one 2-h period per cow). The RC recorded a mean rumination time of 31 ± 5 min/2 h that was similar to the mean rumination time obtained by direct observations 35 ± 6 min/2 h. Using the LoA method (Figure 4.8), an evenly distributed scatter of measurements with no patterns was obtained. No clear tendency was present for the difference between methods to get either larger or smaller as the averages increase. The RC reported rumination

times that were on average 6 min (95% CI –33 to 20 min) shorter than those recorded by direct observations.

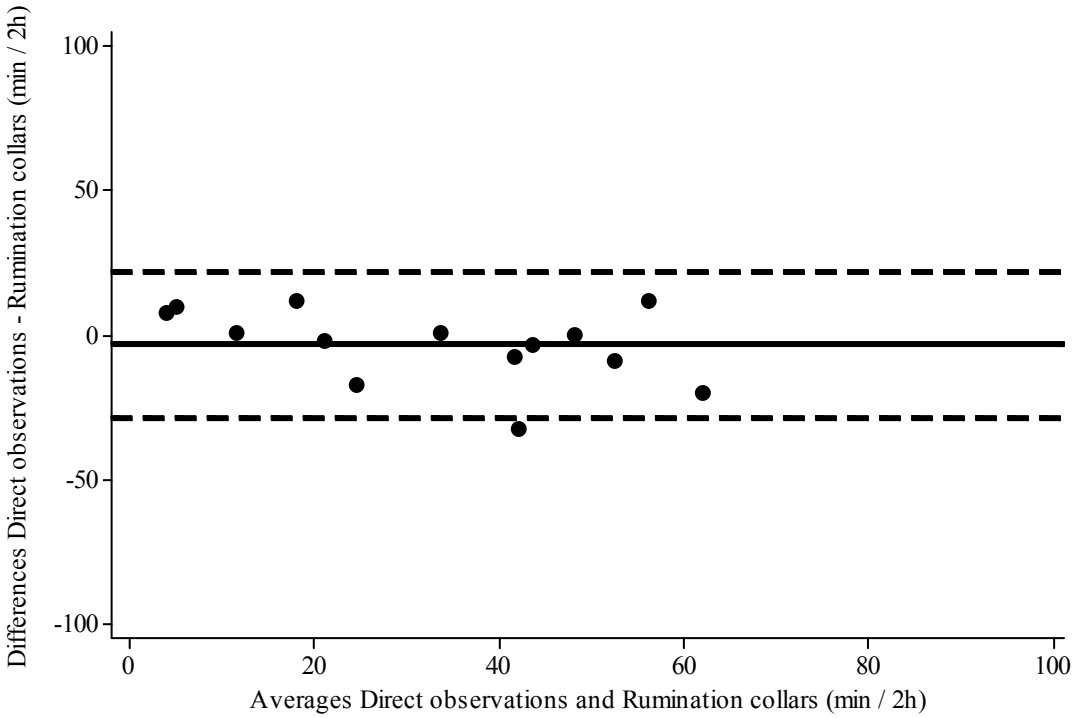

Figure 4.8 The standard Limits of Agreement method, the plot shows rumination time (min/2 h) obtained with the rumination collars and direct observations in Trial 1. A total of 14 2 h periods were recorded from 14 cows. The lines represent the mean difference between the two methods (central horizontal solid line – 3 min) and the limits of agreement (broken lines) higher (upper horizontal line 22 min) and lower (lower horizontal line -29 min).

The standard regression analysis showed a positive relationship (P = 0.001, Figure 4.9), with the slope very close to 1 (slope = 1.08, Table 4.2); when tested, the slope was not different from 1 (P = 0.71).

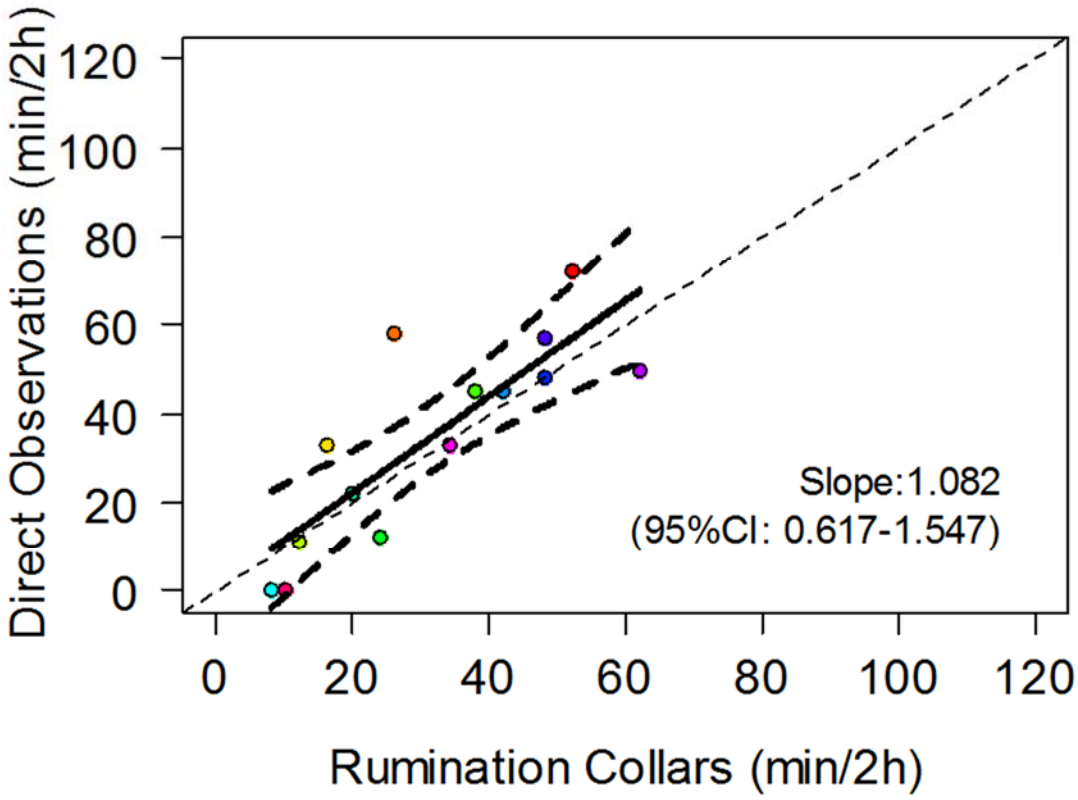

Figure 4.9 Relationship between rumination time (min / 2 h) measured by rumination collars and direct observations in Trial 1. A total of 14 2 h periods were recorded from 14 cows. The broken line depicts the line of equality on which all points would lie if RC and direct observations gave exactly the same reading every time. The solid line shows the equation line and the broken thicker lines show the 95 % confidence interval. Each coloured dot represents a cow from which the recording was taken.

In Trial 2, behaviour was recorded for twenty eight 2-h periods (two 2-h periods per cow). The RC recorded a mean rumination time of 28 ± 4 min/h that was similar to the mean rumination time obtained by direct observations 35 ± 4 min/2 h. The modified LoA method resulted in an evenly distributed scatter of measurements with no patterns or tendencies for the difference to get bigger or smaller as the averages increase (Figure 4.10). The RC reported rumination times that were on average 3 min (95% CI – 32 to 20 min) shorter than those recorded by direct observations.

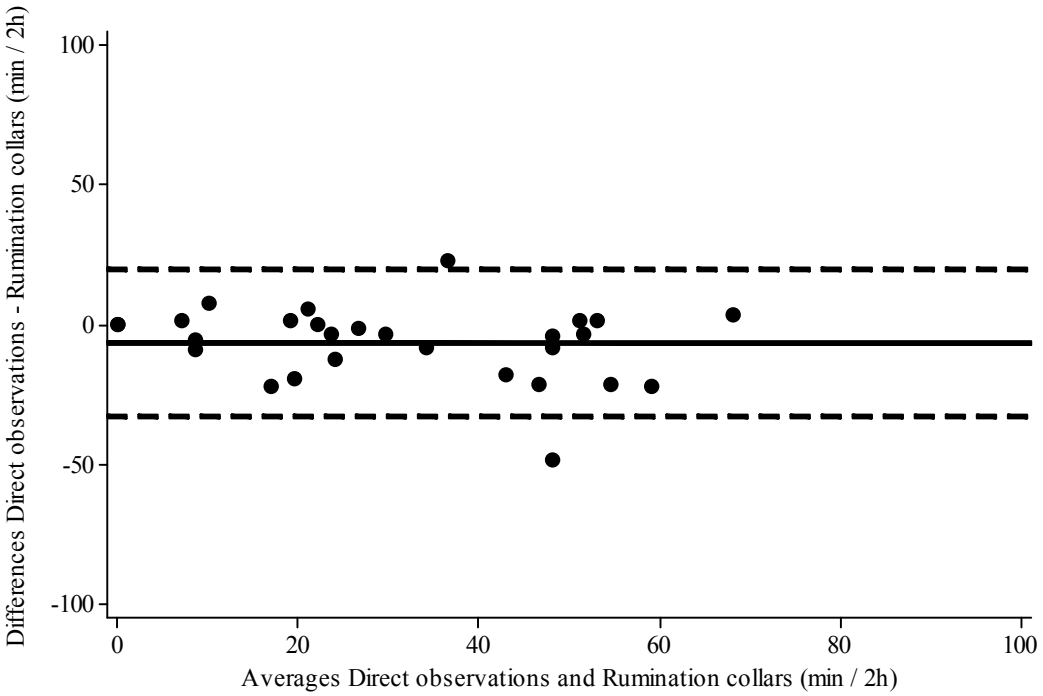

Figure 4.10 The Limits of Agreement plot with multiple observations per individual cow shows rumination time (min/2 h) obtained with the rumination collars and direct observations in Trial 2. A total of 28 2 h periods were recorded from 14 cows. The lines represent the mean difference between the two methods (central horizontal solid line – 6 min) and the limits of agreement (broken lines) higher (upper horizontal line 20 min) and lower (lower horizontal line -33 min).

As with Trial 1, a significant positive relationship was observed ($P < 0.001$, Figure 4.11), with the slope close to 1 (slope = 0.93, Table 4.2); the slope was not different from 1 ($P = 0.63$).

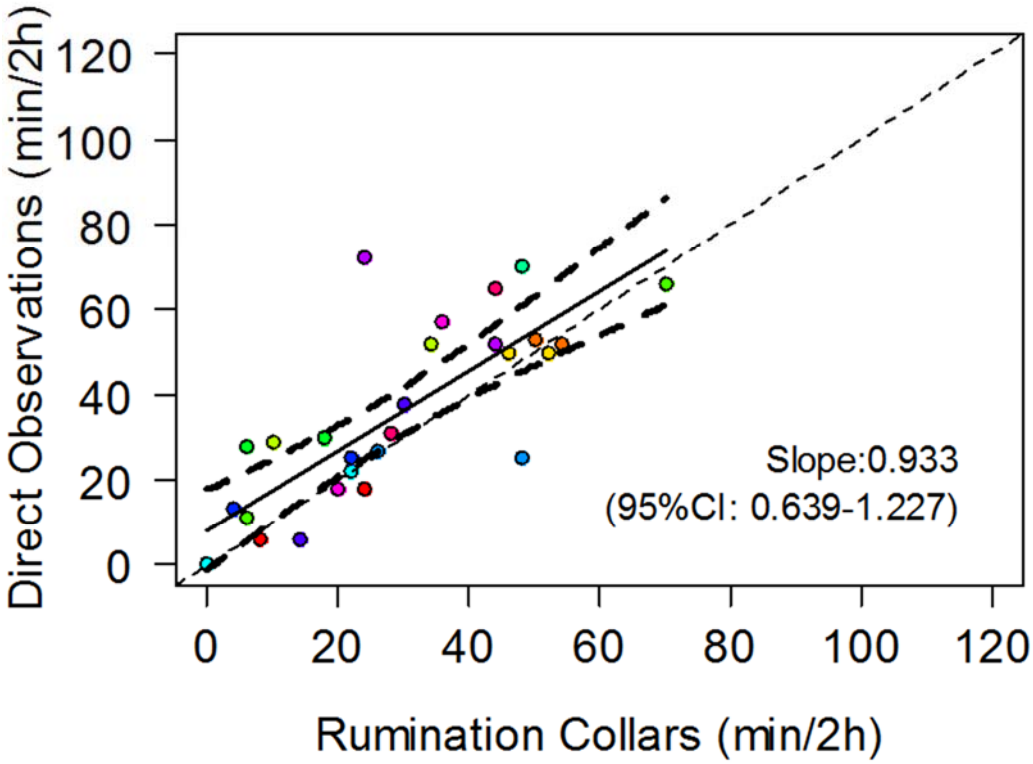

Figure 4.11 Relationship between rumination time (min / 2 h) measured by rumination collars and direct observations in Trial 2. A total of 28 2 h periods were recorded from 14 cows. The broken line depicts the line of equality on which all points would lie if RC and analysis of video recordings gave exactly the same reading every time. The solid line shows the equation line and the broken thicker lines show the 95 % confidence interval. Dots of the same colour represent recordings made from the same cow.

In Trial 3 behaviour was recorded in twenty eight 2-h periods (two 2-h periods per cow). The RC recorded a mean rumination time of 39 ± 4 min /2 h that was similar to the mean rumination time obtained by direct observations 40 ± 5 min/2 h. As with Trials 1 and 2, the modified LoA method showed a scatter of measurements with no patterns and no tendency for the difference between methods to get larger or smaller as the average values increased (Figure 4.12). However, the differences between RC and direct observations were greater than that observed on Trials 1 and 2 (with the 95% CI being -51 to 53 min, average 1 min longer RC).

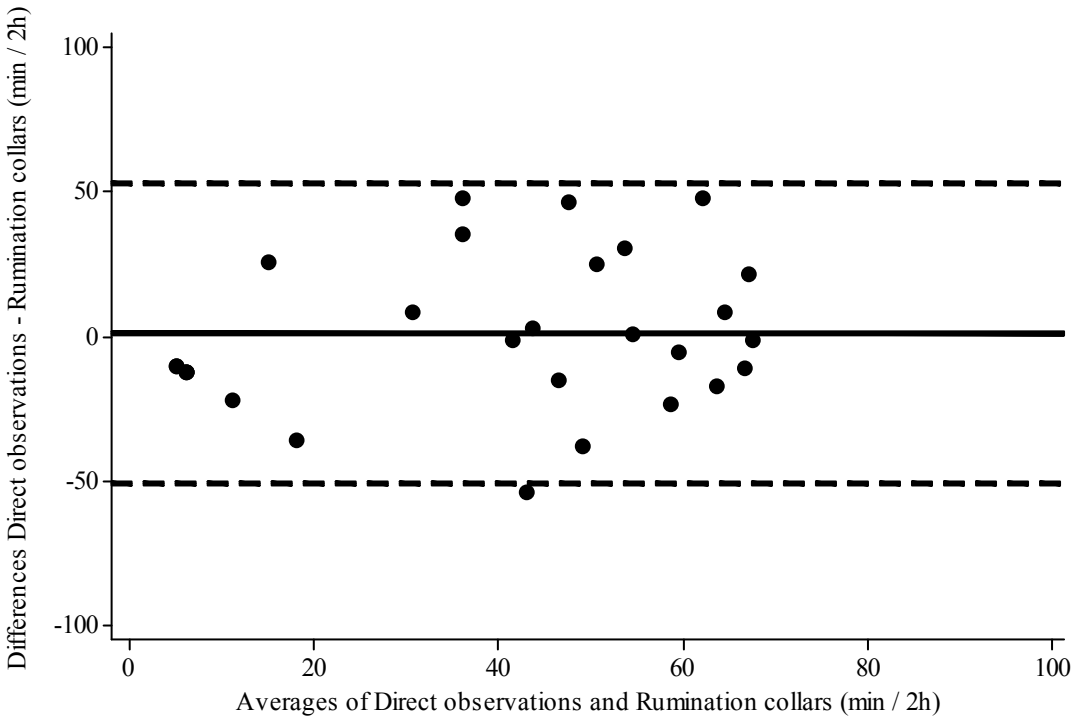

Figure 4.12 The Limits of Agreement plot with multiple observations per individual cow shows rumination time (min/2 h) obtained with the rumination collars and direct observations in Trial 3. A total of 28 2 h periods were recorded from 14 cows. The lines represent the mean difference between the two methods (central horizontal line 1 min) and the limits of agreement higher (upper horizontal line 53 min) and lower (lower horizontal line – 51 min).

A significant positive relationship (P = 0.02) was observed between visual observation and the RC (Figure 4.13). In contrast with Trials 1 and 2, in Trial 3 the slope of this relationship was far from 1 (slope = 0.57, Table 4.3). However when tested statistically, the slope was not different from 1 (P = 0.06).

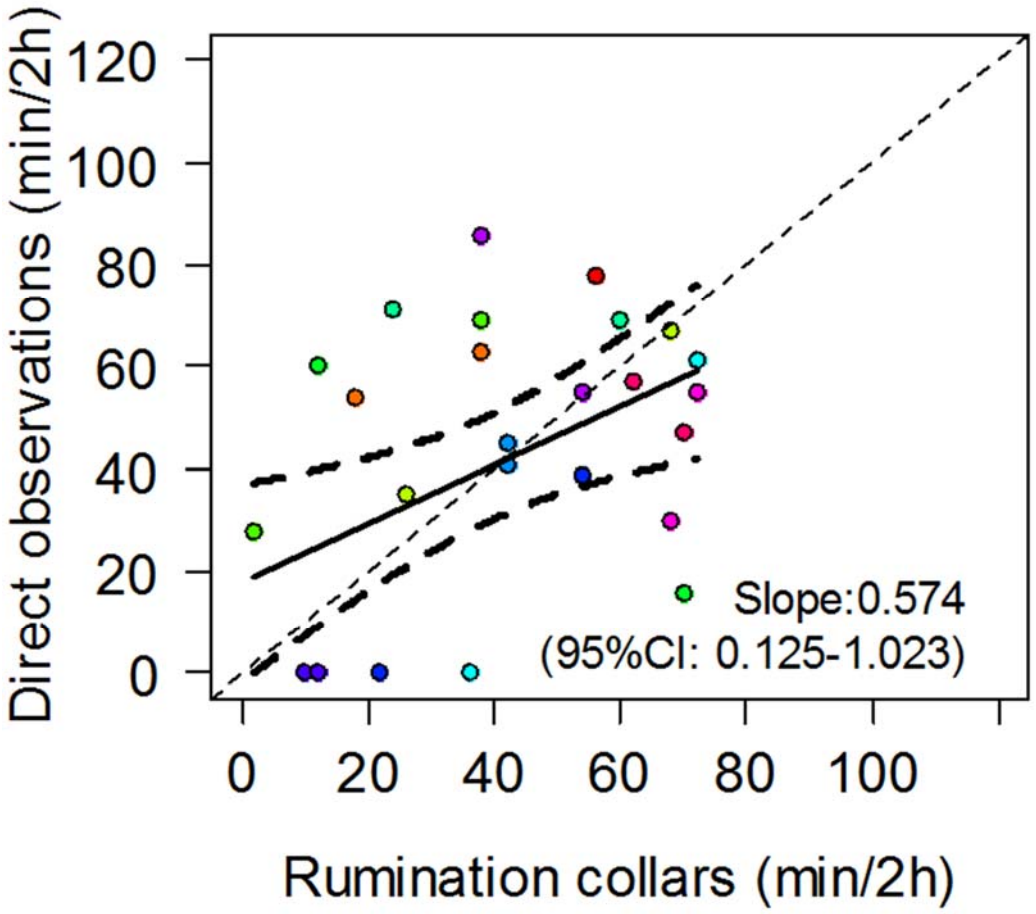

Figure 4.13 Relationship between rumination time (min / 2 h) measured by rumination collars and direct observations in Trial 3. A total of 28 2 h periods were recorded from 14 cows. The broken line depicts the line of equality on which all points would lie if RC and analysis of video recordings gave exactly the same reading every time. The solid line shows the equation line and the broken thicker lines show the 95 % confidence interval. Dots of same colour represent recordings made from the same cow.

4.4 Discussion

An accurate and reliable measure of rumination time was obtained by analysis of video recordings with acceptable observer reliability. The observer reliability was similar or even higher than studies in which observers scored rumination time either with direct observations (Elischer et al., 2013; Goldhawk et al., 2013; Schirmann et al., 2009) or from video recordings (Goldhawk et al., 2013).

These results present the first evaluation of the RC under commercial farm settings for both cows housed indoors and for cows grazing grass at pasture, and using a measurement of rumination time by visual observation directly or by analysis of video recordings. It differs from previous evaluations of the RC in that others used controlled settings, by isolating the animals in individual pens to then be observed (Schirmann et al., 2009), or did not use known values of rumination behaviour (Byskov et al., 2014). Also in their previous validation of the RC, Schirmann et al.(2009) and Elischer et al. (2013) reported problems with accurately recording rumination due to the inability of detecting the start and finish of each rumination bout, or due to the fact that the cow's head was not visible to the observer at a distance.

In this study, such problems were not an issue. For the analysis of video recordings, only 2-h periods were used when it was possible for the observer to detect the start and finish of the rumination event and when the cow was visible; time slots that did not comply with this were eliminated. Three weeks before the start of the recordings by direct observations, cows were accustomed to the presence of the observer. Furthermore the observer was able to determine the start and end of the rumination bout at all times from a distance far enough away as to avoid affecting the cow's natural behaviour (i.e., changing current behaviour or moving away from the observer). Although the rumination time recorded by analyses of video recordings and the RC were highly correlated, variations between individual cows were observed.

Our results were similar to those obtained on previous validations of the RC with recorded rumination times varying from 0 to 90 min/2 h (Elischer et al., 2013;

Schirmann et al., 2009). The variations on the performance of the RC could be explained by differences between cows: for example, thicker skin that interfered with the microphone, differences in movement that misplaced the RC from the neck, or variation in behaviour when ruminating could have affected the RC data (Elischer et al., 2013; Goldhawk et al., 2013).

The rumination time recorded by direct observations and the RC was highly correlated in Trials 1 and 2. However for Trial 3, the relationship was poor as the slope was far from 1. The results obtained from the indoor Trials were very similar when comparing analysis of video recordings and direct observations with the RC. All the indoor Trials showed data sets with narrow confidence intervals, a tight scatter of dots, and an equation line with a slope very close to the line of perfect agreement. The results obtained in Trial 3 with cows outside grazing however showed poor agreement between the RC and the direct observations data set, as indicated by wider limits of agreement (–51 to +53 min) shown by the LoA method, a wider scatter of dots with wider confidence intervals, and a slope far from 1.

This finding is similar to previous work (Elischer et al., 2013), where differences between the 2 measurements of up to 50 min/2 h were recorded, and the RC on average recorded shorter (up to 50 min/ 2 h) rumination times compared to visual observations.

In general, although no marked tendency was observed, it is nonetheless noteworthy that on several observations, the RC reported rumination time (1 to 25 min/2 h) when nothing was recorded by the observer (Figures 4.7, 4.9, 4.13). Similar results have been reported for the RC used with dairy (Elischer et al., 2013) and beef cattle (Goldhawk et al., 2013). This could be explained by malfunctions in one or more of the RC, or by the fact that positioning of the RC changed due to the free movement of the cows around the pen. Furthermore activities such as licking and self-grooming, drinking and other background noises such as rain and wind (especially when cows are at pasture) could have interfered with the recordings made by the RC's microphone. However no relationship was observed in this study when data from

Trial 3 were analysed combining multiple behaviours together such as rumination and eating, or rumination and drinking with the RC output data.

Outdoor farm environments inevitably introduce some level of background noise into a recording, and it can be variable and unpredictable (Navon et al., 2013). This background noise could be the cause of errors in the RC when recording rumination, and cancelling noise technology could be used to improve the RC performance. Possible malfunctions of the RC are not easily detected because there is no standard method to determine if the RC is functioning correctly and that its position on the cow's neck is correct at all times. An alternative to correct and control the position of the tag on the cow's neck could be the use of a halter instead of a collar.

Measurements of rumination time obtained with RC proved to be acceptable under the conditions of Trials 1 and 2, conducted with animals housed indoors. These results suggest that the use of the RC in commercial farms can be advised for the determination of rumination activity and could be an alternative to visual observations for indoor housed cows. However the RC performance when used with cows on pasture grazing was poor. The use of the RC on cows grazing grass should not be advised until further research and validation is carried out.

Further research is now needed to determine key issues for the use of the RC under practical commercial farm conditions. For example it is not known how many cows need to be monitored each day using the rumination collars to give a representative assessment of the herd (including how many cows need to be monitored using a RC in each group, their physiological status, or stage of lactation). Furthermore to be able to use rumination activity as an indicator or proxy measure of health and welfare, it is necessary to assess the effect that physiological factors such as parity, stage of lactation (DIM), pregnancy, and oestrus have on rumination. And lastly more research is needed to determine how management and environmental factors such as diet, number of milking, and temperature (heat stress) affect rumination.

Once these relationships have been explored, it could be possible to define thresholds to assess rumination time on farm, and determine what should be regarded as "normal" or "optimum" rumination time, and what variation from this measure would be consider a problem where intervention was required.

Chapter 5 Results

Assessment of rumen pH dynamics in cubicle housed and grazing dairy cows.

5.1 Introduction

Rumen pH is highly important for the health, welfare and performance of dairy cows. Alterations from rumen homeostatic equilibrium will compromise not only rumen ecology but also nutrition i.e. digestion and available nutrients for the host (Dijkstra et al., 2012). Fermentation of feedstuffs produces VFA and lactic acid, and rumen pH declines when these products accumulate in the rumen. Mechanisms to prevent this include removal of acids from the rumen (by passage in the liquid phase or absorption through the rumen wall) or buffering of rumen pH (by saliva or buffers added to the diet). Other factors that influence rumen pH include dietary components: on the one hand, high levels of rapidly fermentable carbohydrates in the diet such as starch and/or sugars will result in high amounts of VFA which (without sufficient buffering capacity) will reduce rumen pH. On the other hand, NDF and peNDF increases chewing activity, which increases salivary buffer production hence increasing rumen pH levels.

Due to its importance, previous research efforts have developed different methods of measurement of rumen pH, including rumenocentesis, oro-gastric tube, rumen fistula and indwelling rumen probes or sensors.

Two major challenges for collecting and measuring rumen pH are that rumen pH is not homogeneous (or constant) within the rumen, and that different sampling techniques will produce different results. Duffield et al. (2004) observed differences in rumen pH measurements obtained by two different methods. Rumen fluid obtained from the ventral sac of the rumen via cannula against that obtained via rumenocentesis showed 0.33 units of pH difference. When rumen fluid samples were obtained using a stomach tube, measurements were on average 0.35 units higher than the pH of rumen fluid samples collected by rumenocentesis. The authors speculated

that rumen pH obtained from the central rumen had lowest pH values, due to higher concentrations of rumen VFA. Similar results were obtained by Garrett et al. (1999) who found a difference of 0.28 pH units between rumen pH fluid collected via a rumen cannula or via rumenocentesis, with the lower values recorded using rumenocentesis. In a more resent study, Shen et al. (2012) compared the use of an oro-gastric tube and collection via a rumen cannula. The authors found differences between the two methods, which varied depending on the depth at which the tube was located in the rumen to collect the fluid sample. Similar results between the two methods were obtained when the tube was inserted deeper, to reach the central rumen.

The use of continuous monitoring of rumen pH could eliminate the problems in measurement bias detailed above. Furthermore continuous monitoring of rumen pH is advantageous due to its high diurnal variation; it enables the detection of subtle diurnal fluctuations that are difficult or not perceived with single time point evaluations such as those obtained via rumenocentesis.

An alternative to “on-farm” measurements is the use of mathematical models. Such models have been developed based on quantitative understanding and ability to describe the dynamics involved in rumen function, or as a method to evaluate relationships between rumen variables (rumen pH) and other measured variables such as peNDF or VFA. Efforts in modelling rumen function and rumen pH have been put forward from rumen models developed by France et al. (1982), Dijkstra et al. (1992), Lescoat and Sauvant (1995) and more recently Mills et al. (2014) with variable results (Sarhan and Beauchemin, 2015).

To our knowledge, evaluation of rumen pH measurements obtained with rumen pH boluses under commercial farm conditions have not yet been carried out, as the only evaluation has been performed in fistulated animals. The development of mathematical models examining the relationship of rumen pH values obtained with rumen pH boluses and variables measured on-farm has also not been described.

5.2 Evaluation of rumen pH values obtained from rumen pH boluses

5.2.1 Introduction

Subacute rumen acidosis (SARA) is proposed to be a common and economically important problem for dairy cattle. Clinical signs of SARA in a dairy herd include a decrease in DMI, laminitis and diarrhoea (Kleen and Cannizzo, 2012; Krause and Oetzel, 2006; Plaizier et al., 2008).

Assessment of rumen pH has always been challenging due to the fact that methods to obtain rumen fluid are invasive and cumbersome. The use of new technologies to measure physiological, behavioural and production parameters can improve management strategies and performance. The first attempts of using new technologies to continuously record rumen pH were those reported by Dado and Allen, (1993), Nocek, (2002a) and AlZahal et al. (2007b). In a technical note Alzahal et al. (2007b) reported their results on the evaluation of system of continuous ruminal pH recording. Using a fistulated animal, the authors compared the pH values reported by the sensor with those obtained through the fistula. A high correlation was obtained between the two methods ($r = 0.88$ $P < 0.005$). It is noteworthy however to mention that samples of rumen fluid were obtained from the same area within the rumen (adjacent one another). Furthermore the rumen pH measurement system was re-calibrated regularly throughout the Trial.

The use of radio telemetric wireless boluses to measure rumen pH in ruminants has increased in the last few years, especially in research and higher education institutions (Rutten et al., 2013; Sato et al., 2012a). Mottram et al. (2008) presented the first evaluation of a wireless probe. Using four fistulated animals, the authors compared the measurements recorded with the boluses, and that obtained with a pH meter. Although the aim of their study was to demonstrate the ability of the sensor to accurately record rumen pH for up to 42 days, no statistical analyses were carried out. Using figures detailing the values obtained with both methods the authors concluded that the boluses were capable of recording rumen pH for up to 35 days.

However after that time point the measurements obtained started to diverge. In a similar experiment evaluating a wireless probe, Sato et al, (2012a) found a strong correlation ($r = 0.95$, $P<0.01$) between rumen pH obtained using spot-sampling method and the wireless rumen probe, when pH was recorded from the same part of the rumen (middle) with the two methods. However when the rumen pH values were compared from samples recorded from different areas (the bottom of the rumen) a difference of 0.3 units of pH was observed.

The reliability of the rumen pH bolus measurements is based on laboratory based trials and *in vivo* trials with fistulated animals. However no on-farm validation of the bolus measurements has been performed in non-fistulated animals, particularly over prolonged periods of time when the rumen boluses are not re-calibrated repeatedly prior to measurement (which is only possible in fistulated cows). Therefore the primary aim of the present Chapter was to evaluate the measurements recorded using the WellCow rumen pH boluses in commercial farm settings, and compare them with measurements of rumen pH obtained by rumenocentesis. Using this method, it was possible to check for potential drift in the rumen pH bolus accuracy over the three month manufacturers lifespan of the bolus.

5.2.2 Materials and Method

All procedures related to animals were performed under PPL 70/8105 obtained by the Royal (Dick) School of Veterinary Studies of the University of Edinburgh. The Trial (Trial 4) had a similar structure to that of the previous three Trials, however specific details and differences are detailed as follows.

5.2.2.1 Animals and housing

Six dairy cows were selected primarily by DIM (> 200 DIM). Cows were (329 d ± 46, mean ± SEM) DIM and parity (two 1st lactation, one 2nd lactation and three 3rd lactation plus). All individuals were clearly identified with a unique number by colour spray (Arco Ltd., England UK). At the beginning of the Trial (first two

weeks) the cows were kept indoors (as described in Chapter 3 for Trials 1 and 2). After this period, the animals were outside grazing (as described in Chapter 3 for Trial 3).

5.2.2.2 Experimental Design

The cows were kept under the same housing conditions as described in Chapter 2. The Trial ran for 13 weeks. During the first nine weeks, rumen fluid samples were obtained from each cow via rumenocentesis (as described below section 5.2.2.4) once every two weeks. For the remaining weeks, samples were collected weekly. Each rumen fluid sample was taken at a predetermined time (starting at 08.00 am) so that sampling each cow match the time of the rumen bolus pH recording. The changes in diets and management routines were designed to collect a wide range of rumen pH levels.

5.2.2.3 Diets offered to cows

At the start of Trial 4 when the experimental animals were housed indoors (from 18th of May until 5th of June), cows were offered a PMR consisting of 2nd cut grass silage 48.4 %, wholecrop 16.9 %, Langhill dairy meal 24.2 %, Water 7.3 %, Molasses 3 % and Equaliser with Amferm 0.2 %. After this indoor period, the cows were outside grazing a ryegrass (*Lolium perenne*) sward during the day and night. In addition cows were offered a buffer PMR consisting of 1st cut grass silage 39.3 %, wholecrop 34.4 %, Langhill dairy meal 22.1 %, and Molasses 4.2 %. From July 24th and for the remaining duration of the Trial, the PMR consisted of 1st cut grass silage 44.4 %, wholecrop 22.2 %, Langhill dairy meal 20.0 %, grass silage 1st cut bale 8.9% and Molasses 4.4 %. Details of the diets offered to the cows are shown in Table 5.1

5.2.2.4 Data collection

To record rumen pH, cows were orally administered an intra-rumen bolus (WellCow Ltd., Roslin, Scotland UK) as described in Chapter 3. Prior to deployment, the

boluses were calibrated against known standard pH buffer solutions (pH 4 and pH 7) using an application (App) interface installed on a mobile phone according to the calibration procedure recommended by the manufacturer (WellCow Ltd. Operating Manual V2). The boluses were set to record pH at 15 min intervals for the entire lifespan of the bolus's battery. The recorded data was stored in the bolus's memory, and then transmitted using the App and via radio signal to a receiver connected via Bluetooth to a smart phone. The data was then transferred to a personal computer for further analysis.

Collection of rumen fluid by rumenocentesis was performed as follows: a 10 cm square area located approximately 15 cm caudoventral to the costochondral junction of the last rib on a line parallel with the top of the stifle was identified on the left side of the cow. The area was clipped and aseptically prepared. A stainless steel sterile needle (100 mm 16 gauge, Air Tite Products Co., USA) was inserted into the ventral rumen, and a syringe was used to aspirate 10 to 20 ml of rumen fluid (Garrett et al., 1999; Nordlund and Garrett, 1994). Immediately afterwards the rumen liquor was placed into a plastic container, and rumen fluid pH was measured (within one minute of collection) using a portable pH meter (Hanna Instruments H198127).

Prior to every analysis, the portable pH meter was calibrated using a two point calibration according to the manufacturer's recommendations, using a standardised buffer solution = 4.01 pH followed by a buffer solution = 7.01 (buffers provided by Hanna Instruments). The probe was rinsed with deionised water between measurements. The meter manufacturer claims resolution of 0.01 pH.

5.2.2.5 Calculations and Statistical Analysis

The measurements obtained with the two methods (rumen pH bolus and rumenocentesis) were compared to determine the accuracy of the rumen boluses to measure rumen pH, and to determine the reliability of the measurements across time. To evaluate the relationship between the rumen pH values obtained with the pH meter and the rumen boluses, and to take account of the multiple observations per

individual cow, a modification of the standard Limits of Agreement (LoA) methodology and a standard linear mixed effect model were used. In the linear mixed effect model, which cow that the measurement had come from was entered as the random effect.

All statistical analyses were carried out using R with the linear mixed-effect analysis performed using the nlme package (version 3.1-113), and the modified version of the LoA with repeated measures as modified by Nutter (2008). Statistical significance was taken as $P<0.05$.

5.2.3 Results

Table 5.1 shows the diets offered throughout Trial 4.

Table 5.1 Ingredients and chemical composition of the offered diets.

Composition	Periods		
	Indoors	Outdoors grazing	
Ingredient (kg per cow per day Fresh Weight)	From the 18th May to the 5th of June	From the 6th of June to the 24th July	From the 24th of July
Grass silage 2nd cut	20	8.0	
Wholecrop	7	7.0	5.0
Langhill meal	10	4.5	4.5
Molasses	1.25	0.9	1.0
Water	3		
Equaliser with Amaferm	0.10		
Grass silage 1st cut			10
Grass silage 1st cut bale			2
Grazing (fresh grass)		45	45
Parlour concentrate (fed to yield)	3	3	3
Analysis			
DM (%)	49.7	36.3	36.3
CP (% DM)	16.3	16.5	16.6
NDF (% DM)	34	38	38
uNDF forage (% DM (kg total))	6.5	10.3	9.7
uNDF total (% DM (kg total))	11.0	12.9	12.3
Oil (% DM)	4.5	3.5	3.7
Sugar (% DM)	7.9	7.2	7.6
Starch (% DM)	16.9	11.1	10.1
Quick CHO (% DM)	17.9	18.8	19.5
Slow CHO (% DM)	39.7	40.4	39.4

Table 5.2 summarises the sampling of individual cows during the Trial. At the beginning of the Trial, the bolus in cow 298 failed and so this cow was not sampled

by rumenocentesis. The bolus in cow 11 also failed, and was replaced by a new bolus after 2 weeks into the Trial. Cows 51 and 102 were removed from Trial 4 due to difficulties (resistance posed by the animals, no fluid obtained, etc.) in obtaining rumen fluid samples by rumenocentesis. A total 20 measurements (Table 5.2) were obtained from 5 experimental animals via the rumenocentesis procedure. However due to blood contamination and small quantities of fluid obtained some of these measurements were discarded and only 13 measurements were used for subsequent analysis.

Table 5.2 Rumen samples obtained by rumenocentesis throughout the trial

		Cow id					
Date	Week of study	298	11	51	297	102	117
Tuesday 19th May	1		X				
Tuesday 2nd Jun	3			X	X	X	X
Tuesday 16th Jun	5			X	X	X	X
Tuesday 14th Jul	9		X		X	X	X
Tuesday 28th Jul	11				X		X
Tuesday 4th Aug	12		X		X		X
Tuesday 1th Aug	13		X		X		

Figure 5.1 shows the daily mean rumen pH values recorded with the rumen boluses throughout Trial 4 in two cows (boluses deployed on same day), and illustrates representative data obtained via the rumen pH boluses.

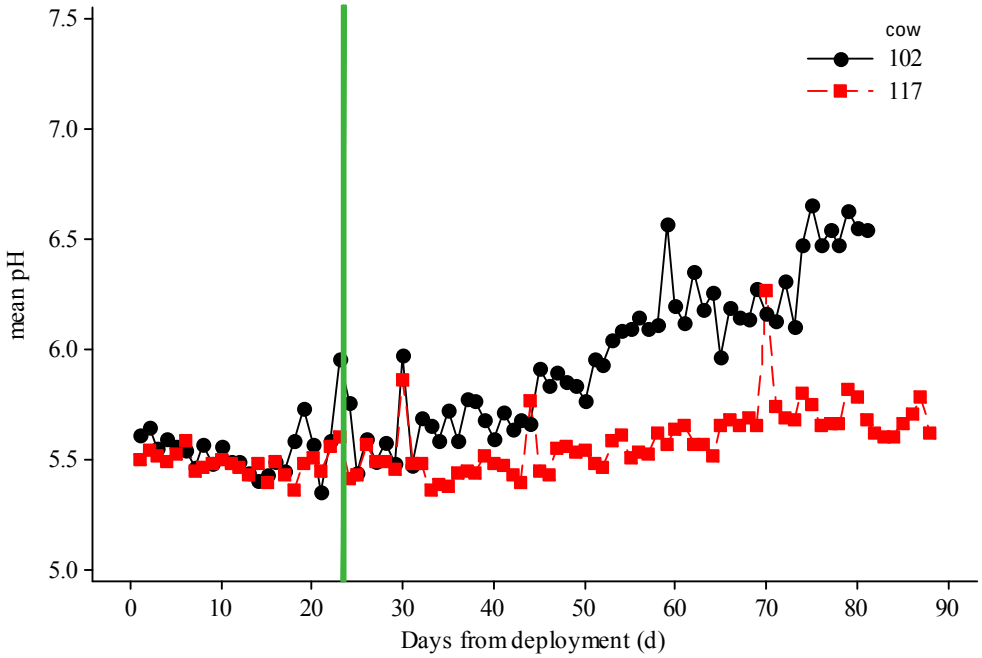

Figure 5.1 Mean daily rumen pH obtained with the rumen boluses in two of the experimental cows. This mean value was calculated from the 96 data points obtained for each day. The green line shows the time of the major diet change, when the cows went out to grazed grass.

Figure 5.2 shows the rumen pH values obtained using the rumenocentesis procedure at the determined sampling time points, while Figure 5.3 shows the rumen pH values obtained with the rumen boluses at the same sampling time points.

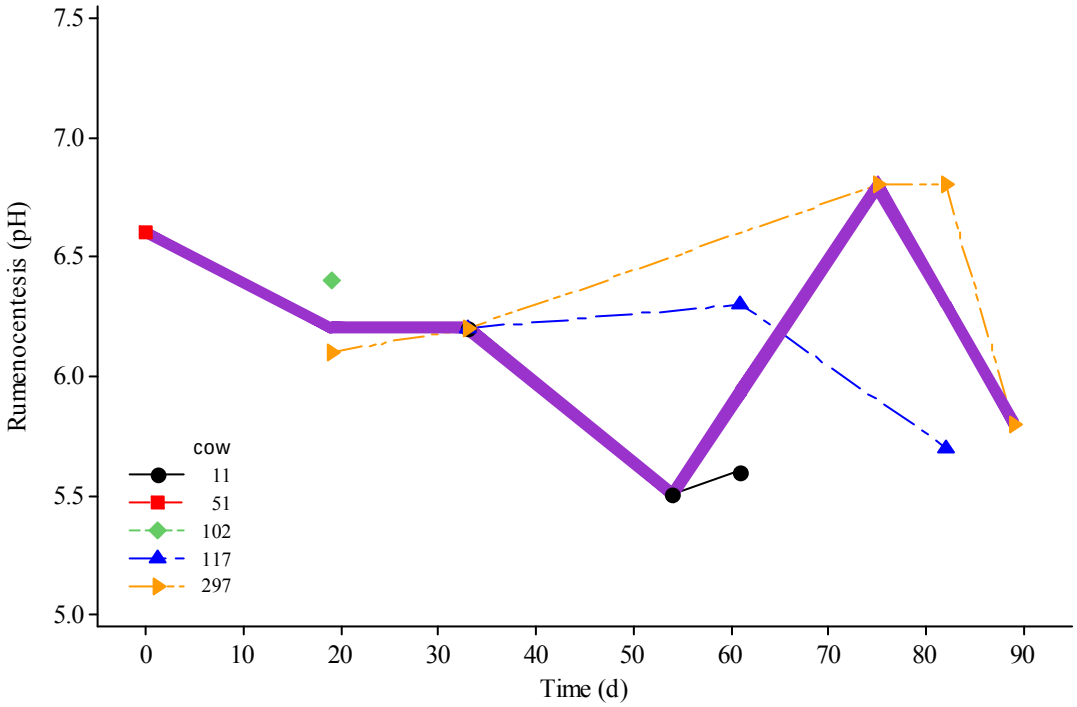

Figure 5.2 Rumen pH values obtained using rumenocentesis, each colour represents an experimental cow and the solid purple line represents the mean of the values recorded on each day.

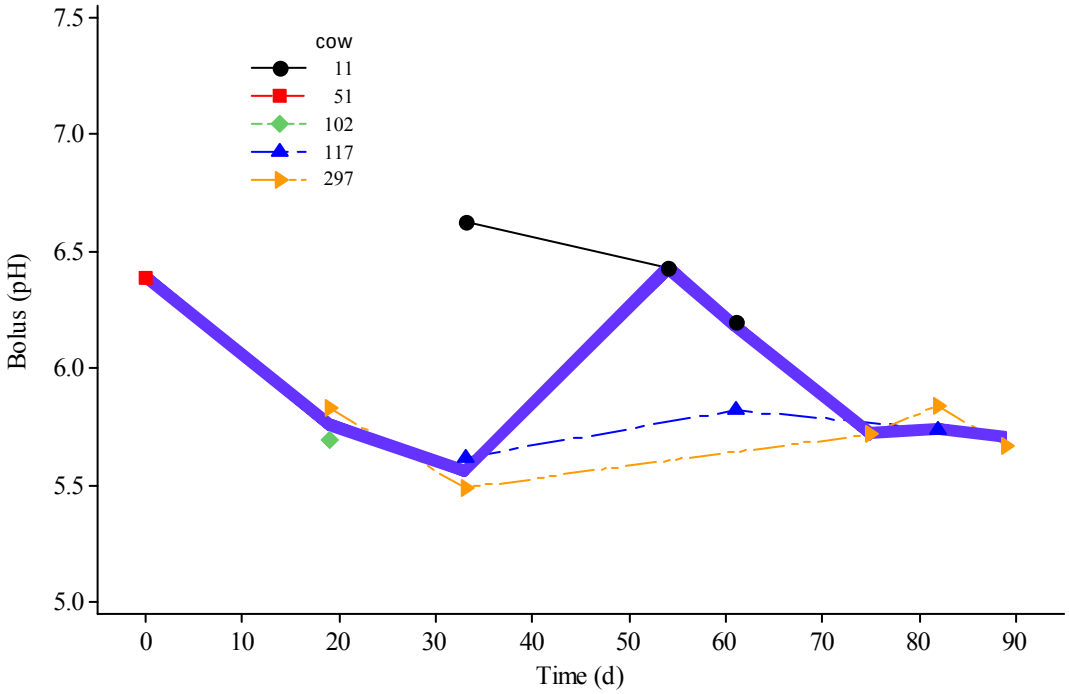

Figure 5.3 Rumen pH values recorded by the rumen boluses, each colour represents an experimental cow and the solid purple line shows the mean of the values recorded.

Figure 5.4 shows the relationship between rumen pH values obtained with the two measurements. There was no significant correlation (r = -0.02 P = 0.52) between the measurements recorded with the two methods (rumenocentesis and rumen pH boluses).

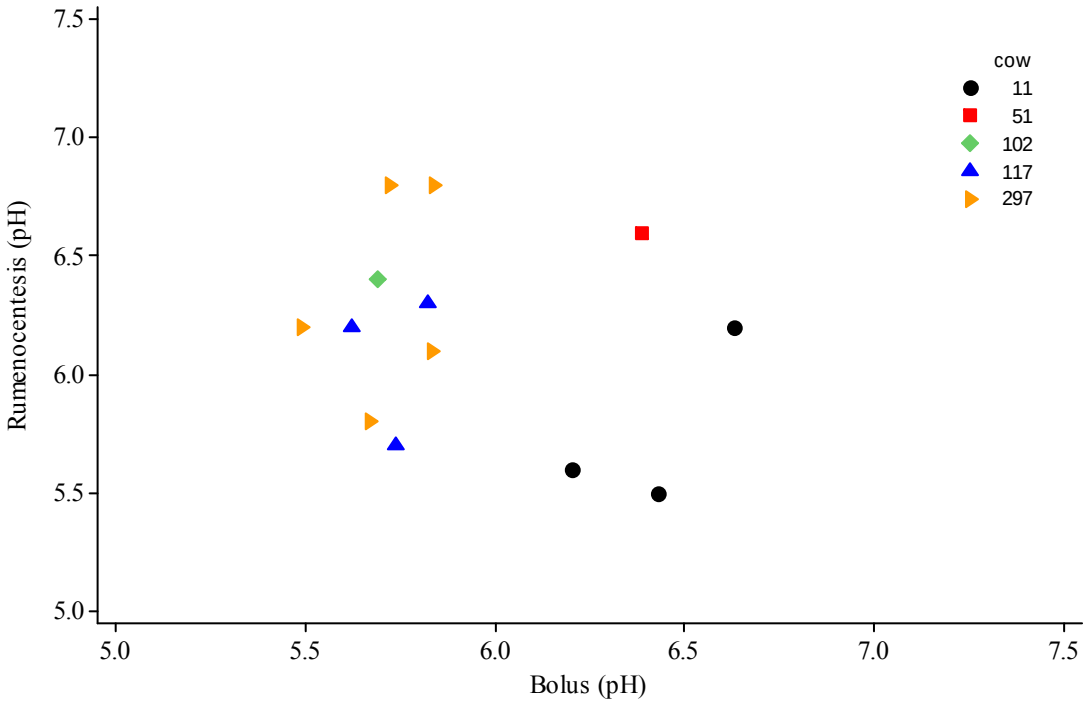

Figure 5.4 Relationship between rumen pH obtained with the two methods (rumen bolus and rumenocentesis) each colour and symbol represent an individual animal.

To explore the relationship between the recorded rumen pH values (obtained with both methods) across time (days from deployment), Figure 5.5 shows the difference between rumen pH values obtained with rumenocentesis and rumen pH boluses at each predetermined time point.

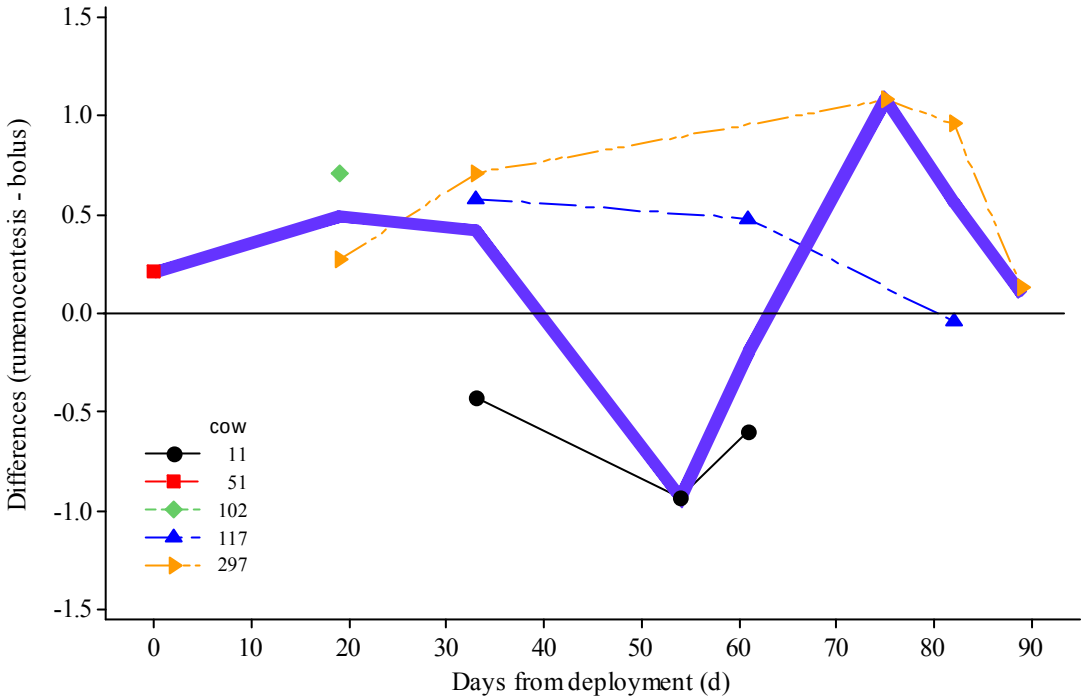

Figure 5.5 Differences (rumenocentesis – bolus) of rumen pH values recorded from experimental cows. The horizontal line represents the line of equality.

Figure 5.6 shows the Limits of Agreement method. The figure shows an evenly distributed scatter of values. However a slight tendency is apparent as the pH values increase, with the differences between the two methods getting larger as the average values increase. The rumen pH values recorded via rumenocentesis were on average 0.24 (95% CI – 0.95 to 1.43) units of pH higher than those recorded with the boluses.

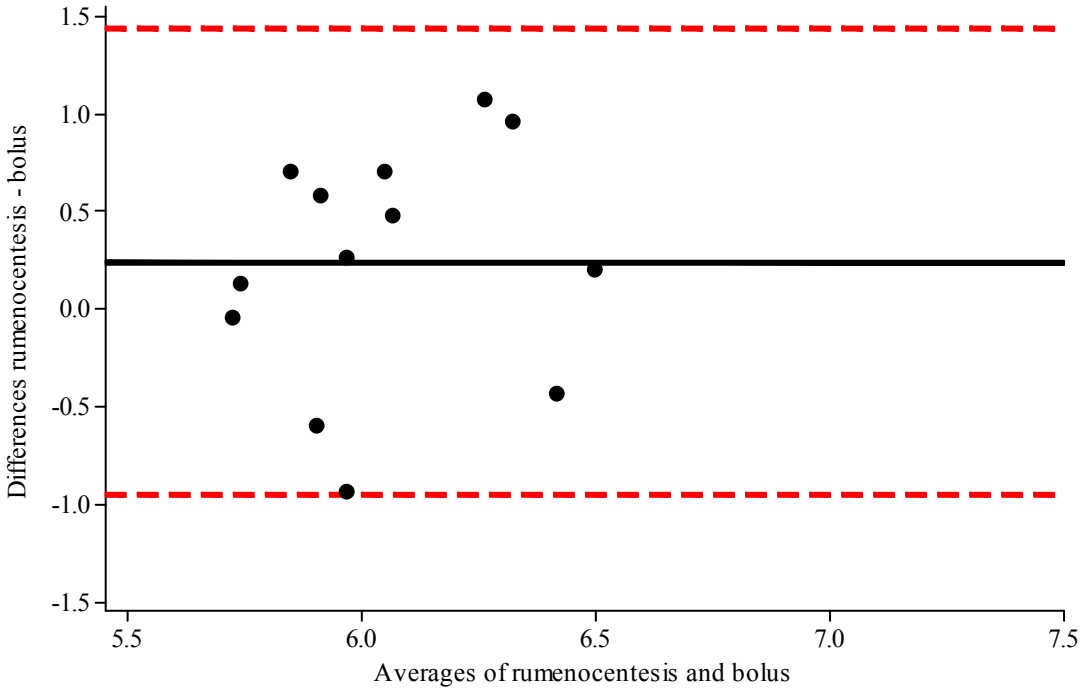

Figure 5.6 The limits of Agreement plot with multiple observations per individual cow shows rumen pH recorded with the rumen boluses and obtained by rumenocentesis. A total of 13 measurements were recorded from 5 cows. The lines represent the mean difference between the methods (central black solid line 0.24) and the limits of agreement higher (upper red broken line = 1.43) and lower (lower red broken line = – 0.95).

5.2.4 Discussion

The aim of the present study was to evaluate the relationship between rumen pH measurements obtained with the use of rumen boluses and rumenocentesis, and to check for potential drift in the rumen pH bolus accuracy over time (over the three month lifespan recommended by the manufacturer).

The results show that there was no significant correlation observed between the two rumen pH measurements, as seen in Figure 5.4.

This lack of correlation could potentially be explained by issues arising from the experimental work. The repeated rumenocentesis procedure was more challenging than expected, and difficulties performing the procedure were experienced throughout the Trial. Two cows sampled suffered from local inflammation reactions, which complicated the sampling technique and resulted in two cows having to be withdrawn from the Trial. Blood contamination was present in some of the samples, which were discarded from the dataset as the presence of blood could alter the pH of the sample. There were also technical issues with the rumen pH boluses during the first half of the trial, caused by the launch of a new version of the software application (WellCow app) used to download the data, malfunction of the mobile phone used to connect and download the rumen pH measurements, and malfunctions of the pH sensors in two of the rumen boluses.

Even allowing for these experimental issues, there may be other potential explanations for the differences arising as result of the sampling procedure and site. As discussed in Section 5.1, variation in rumen pH values have been reported relative to sampling site (Sato et al., 2012a; Shen et al., 2012) and sampling methods (Duffield et al., 2004). It is of note that the 0.24 unit of pH difference observed between the rumen pH bolus and rumenocentesis samples in Trial 4 was similar to that reported by these previous studies. Given that the rumen pH bolus is thought to reside in the reticulum, whereas the rumenocentesis samples would have been taken from the caudal ventral sac of the rumen, such physiological differences might

explain why the rumen pH bolus recorded lower pH values compared to those obtained via rumenocentesis.

Taking these potential explanations for the 0.24 unit of pH differences into account, there was no evidence of "drift" in the measurements of rumen pH values recorded with the rumen boluses over time was observed (Figure 5.5). The differences observed in Trial 4 between the two methods for the assessment of rumen pH would suggest issues with the experimental methodology rather than drift of the output (i.e. changes in the pH calibration of the boluses over time) from the rumen pH boluses *per se*.

However further work is needed to elucidate the accuracy and precision of the rumen pH boluses, and the potential for changes in the accuracy of the pH boluses output over time in non-fistulated animals when it is not possible to re-calibrate the boluses. However in order to do this, rumen fluid sample would need to be taken from the vicinity of the rumen pH boluses. This would potentially require surgical procedures (for example placement of an indwelling rumen catheter) so that the site of pH sampling was accurately known. However technical difficulties, cost and availability of experimental resources would be significant issues with such experiment.

5.3 Rumen pH data analyses

5.3.1 Introduction

Mathematical models allow the prediction of different outputs from animal production without carrying out experiments. The models used can be classified as either statistical (empirical) or dynamic mechanistic models (Thornley and France, 2007). Empirical models have been used to predict many outputs from nutrient or animal characteristics including DMI, milk yield, lactation potential (Friggens et al., 1999; NRC, 2001), and methane output (Ellis et al., 2007).

The ability to predict rumen pH is important to estimate it effects on ruminal digestion of fibre and microbial protein synthesis, and to avoid the occurrence of SARA (Dijkstra et al., 2012). Rumen pH can be predicted from the rumen concentration of volatile fatty acids (Tamminga and Vanvuuren, 1988) or feed characteristics. In a recent review, Sarhan and Beauchemin (2015) evaluated different equations that predict rumen pH from feed characteristics (fibre content; (Fox et al., 2004; Mertens, 1997; Pitt et al., 1996; Zebeli et al., 2008)) or from concentration of VFA (Allen, 1997; Lescoat and Sauvant, 1995; Tamminga and Vanvuuren, 1988) in dairy and beef cattle. The authors concluded that the ability of the evaluated models to predict rumen pH was low, with the best evaluated models (Fox et al., 2004; Pitt et al., 1996) failing to predict values at the extremes of the observed values (over-predicting for low rumen pH i.e. 5.49 predicted versus 5.06 observed and under-predicting for alkaline pH i.e. 6.46 predicted versus 7.09 observed).

The authors concluded that further investigations should use rumen pH predictions from continuous measurements of rumen pH, and with data that included characteristics other than NDF. The best model resulting from this evaluation was that of Pitt et al. (1996). Although limited and with relative low accuracy, the model (as with other empirical models) may help shed some light into the relationship of

rumen pH with some easily measurable variables. This is where the importance of empirical models resides. Therefore this Chapter aims to improve the prediction of rumen pH from data collected on farm, to establish quantitative relationships between different animal and feed characteristics, and predict rumen pH by means of statistical modelling using mixed effect models.

5.3.2 Materials and Methods

5.3.2.1 Data sourcing

A database was constructed using data recorded from Trials 1 – 3 performed at the University of Edinburgh at Langhill Dairy Farm, (Roslin, Midlothian, Scotland UK) during 2012 and 2013. The three Trials are described in detail in Chapter 3, and a brief outline is given as follows.

In its origins, the Trials were designed to evaluate the effect of yeast supplementation on performance, rumination time and rumen pH under different farm environments. In each Trial, fourteen multiparous milking cows (unique to each Trial) were selected and balanced for DIM and parity. The cows were then randomly allocated to two different groups of seven cows to facilitate management routines e.g. milking and feeding. The Trials were divided into experimental Periods for yeast supplementation or not. In each Period cows were given an adaptation time (two to three weeks), and all measurements were recorded in the last week of each Period.

Cows were offered a PMR with additional concentrate fed to yield in the milking parlour. In Trials 1 and 2, cows were housed in cubicle shed. In Trial 3, cows were grazing a ryegrass (*Lolium perenne*) sward during the day and night, and cows were also given a buffer PMR ration for two hours after afternoon milking.

Data on animal performance (milk yield and composition, BCS and BW), behaviour (rumination time in Trials 1 and 2) and rumen environment (rumen pH) was recorded. Feedstuffs were analysed for chemical characteristics (Table 5.3) as described in detail in Chapter 3.

Table 5.3 Variables recorded in the on-farm trials

Factor	Trial 1	Trial 2	Trial 3
Mean rumen pH	X	X	X
Time spent under SARA pH<6.2	X	X	X
Animal			
Parity	X	X	X
DIM	X	X	X
BW	X	X	X
BCS (1 – 5)	X	X	X
Milk yield (kg/d)	X	X	X
Butter fat	X	X	X
Protein	X	X	X
Lactose	X	X	X
Rumination (min / d)	X	X	NA
Feedstuff			
Diet offered	PMR+Conc	PMR+Conc	Grazing+BPMR+Conc
Dry matter	X	X	X
CP	X	X	X
NDF	X	X	X
uNDF	X	X	X
Sugar	X	X	X
Starch	X	X	X

PMR = partial mixed ration, Conc = parlour concentrate, BPMR = buffer partial mixed ration. NA = Not Available

5.3.2.2 Database construction

To explore the relationship between rumen pH, animal and feed characteristics, a database was created from complete available data. From the recording days on each measurement week for each Trial, complete datasets for all the required variables were obtained and collated into one datapoint per animal per day e.g. on the measurement week of Period 1 for Trial 1 on Monday: Milk yield, milk characteristics, rumination time, rumen pH were recorded. For BCS, BW and feed characteristics, values were recorded once a week and were taken as the same values for the whole week (Table 5.4).

Table 5.4 Data collected during the measurement week in each Trial.

Groups	Period	
	Adaptation	Measurements / recording
Group 1 and 2		Milk yield daily (seven days a week) Rumination daily (min/2h or min/24h seven days a week) Rumen pH every 15 min (24h / seven days a week) Milk composition once on Mon, Wed and Fri BCS and BW once a week Feed sampling once a week

Mon = Monday, Wed = Wednesday and Fri = Friday.

5.3.2.3 Calculations and Statistical Analysis

Before collation, all variables in the dataset were checked. Outliers and unreliable data (as defined in Chapter 3 Section 3.3.5) were removed from the dataset.

To explore the relationship between animal, dietary factors and rumen pH, the dataset was subjected to mixed effect model analysis, considering the random effect of each individual cow. The model simplification procedure (MSP) proposed by Crawley (2013) was carried out. A model including all the recorded variables thought to have an effect on rumen pH was constructed. Using the *summary* function, the model was evaluated and the least significant term was discarded using the *update* function. Comparison of obtained and previous models was performed using Analysis of Variance (*ANOVA*) with the maximum likelihood method (*ML*) (Crawley, 2013). The analyses were carried out using R with the mixed effect model analysis and simplification performed using the nlme package (version 3.1-113). Statistical significance was taken as $P < 0.05$.

5.3.3 Results

5.3.3.1 Model creation

A dataset from all three Trials (Trial 1, 2 and 3, Chapter 3) was obtained. Table 5.5 shows descriptive statistics of the variables evaluated.

Table 5.5 Summary of variables recorded

Factor	Mean	SD	Range
Mean rumen pH	6.15	0.24	5.41 – 6.58
Time spent under SARA pH<6.2	735	450.4	0 – 1440
Time spent under SARA pH<5.8	208	351.0	0 – 1440
Animal			
Parity	4	1.38	3 – 8
DIM	177	46	94 – 293
BW	680	47.43	572 – 814
BCS (1 – 5)	2	0.5	1.5 – 3.25
Milk yield (kg/d)	33	7	14 – 50
Butter fat (%)	4	0.67	2.74 – 6.36
Protein (%)	3	0.28	2.41 – 4.18
Lactose (%)	4	0.21	3.66 – 4.74
Rumination (min / d)	466	86.88	248 – 638
Feedstuff (TMR)			
DM	44	9.16	28.30 – 56.70
CP	16	1.08	13.60 – 16.80
NDF	37	3.62	33.00 – 43.00
uNDF total	13	2.49	9.90 – 17.70
Sugar	7	1.45	5.30 – 9.70
Starch	15	4.52	5.80 – 19.80

Rumen pH was defined as mean daily pH, and also as time (min/d) spent under SARA threshold (pH<6.2). This was used to provide a better representation of the highly dynamic pattern of rumen pH values observed across the dataset. A mixed effect model was then constructed for each of these variables i.e. mean pH (lmemeanpH) and time under SARA (lmemin62).

The maximal model for each of the variables included: Trial (Trial 1, 2 or 3), animal characteristics (parity, DIM, BW, BCS, milk yield, butterfat, protein content, lactose content and rumination time) and feed characteristics from TMR (DM, CP, NDF, uNDF, Sugar and Starch). However due to issues with the unbalanced nature of the dataset, and a high correlation between feed characteristics observed (NDF, Starch and Sugar; see Figures 5.6 and 5.7), it was not possible to run the maximal model.

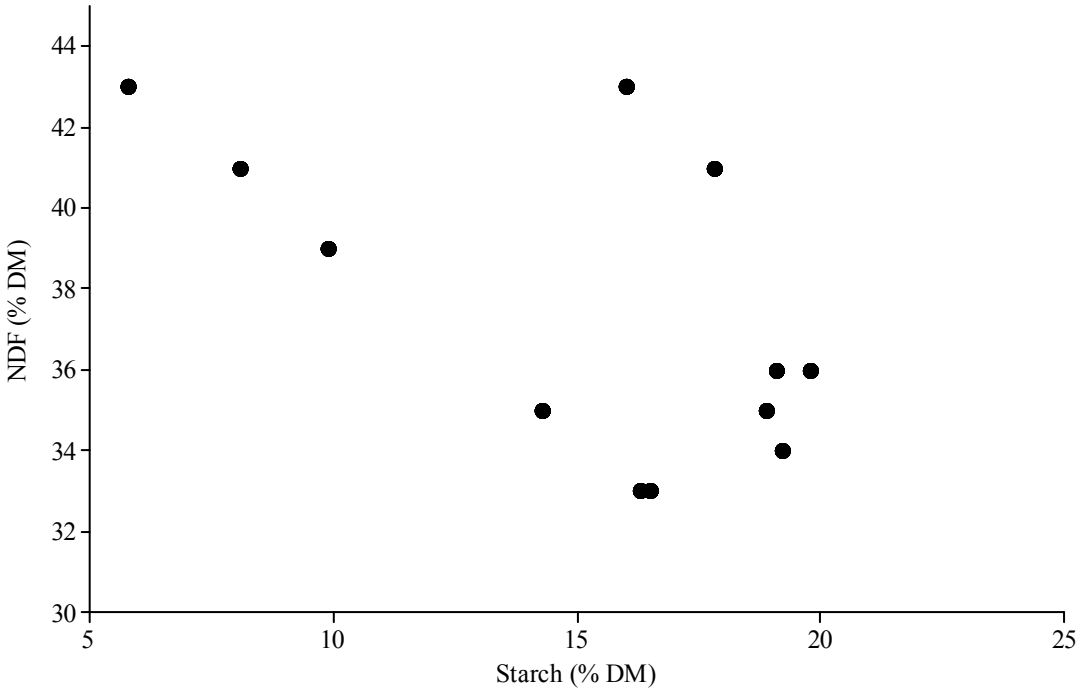

Figure 5.6 Relationship between NDF and Starch content recorded in the three trials.

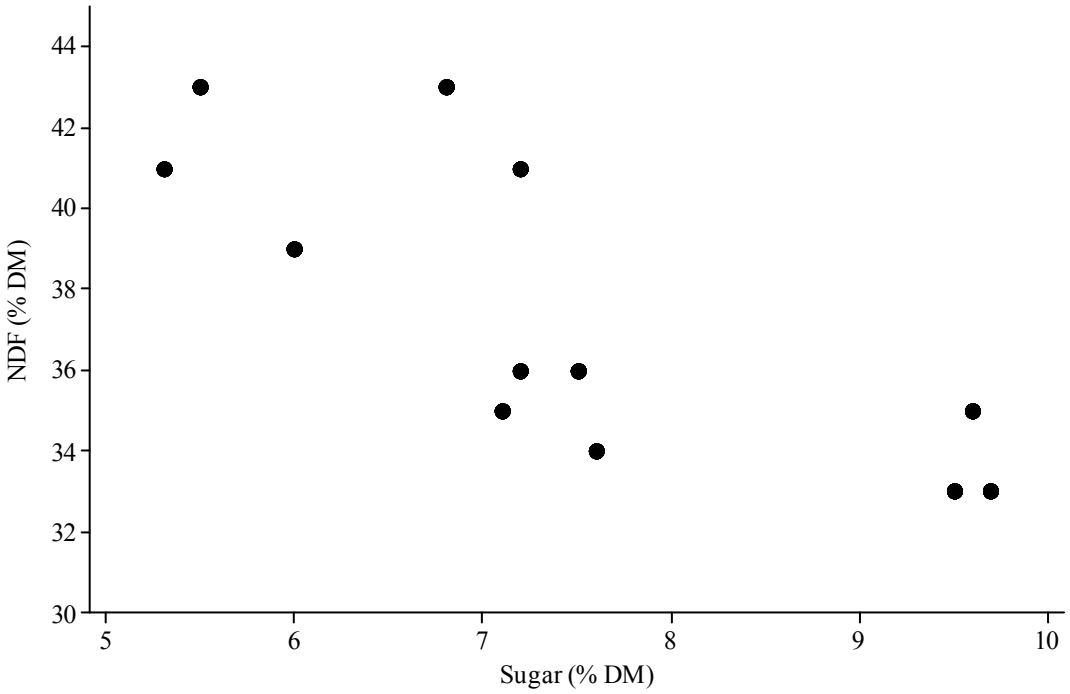

Figure 5.7 Relationship between NDF and Sugar content recorded in the three trials.

The MSP was carried out until all the remaining factors were significant. The significance of each variable and its p value at each of the MSP steps are presented in Table 5.6 for lmemeanpH and in Table 5.7 for lmemin62.

A first evaluation of the two obtained models (lmemeanpH and lmemin62) was to compare the minimal adequate model with the maximal model and intermediate models developed during the MSP. No statistically significant differences between these models were observed (P>0.05) i.e. including all the available variables to the maximal model did not improve the prediction compared to the minimal model.

Table 5.6 Model simplification process, significance values per each individual component of the lmemeanpH model for mean rumen pH.

Variable	p value										
	all	-BW	-Rum	-Lact	-BCS	-NDF	-MY	-Prot	-Parity	-Bfat	-DM
Animal											
Parity	0.04	0.05	0.20	0.40	0.42	0.49	0.48	**0.50**			
DIM	0.19	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BW	**0.89**										
BCS	0.02	0.01	0.95	**0.95**							
MY	0.47	0.45	0.66	0.65	0.73	**0.73**					
BF	0.01	0.01	0.41	0.41	0.57	0.58	0.57	0.35	**0.45**		
Prot	0.48	0.44	0.42	0.41	0.56	0.55	**0.62**				
Lact	0.40	0.38	**0.99**								
Rum	0.67	**0.68**									
PMR											
DM	0.20	0.20	0.04	0.04	0.08	0.07	0.07	0.07	0.05	**0.05**	
CP	0.00	0.00	0.07	0.07	0.15	0.05	0.05	0.04	0.02	0.02	0.04
NDF	0.10	0.10	0.88	0.88	**0.88**						
uNDF	0.11	0.08	0.67	0.67	0.30	0.29	0.31	0.33	0.34	0.01	0.01

DIM = days in milk, BW = body weight, BCS = body condition score, MY = milk yield, BF = butterfat, Prot = protein, Lact = lactose, Rum = rumination activity, PMR = partial mixed ration, DM = dry matter, CP = crude protein, NDF = neutral detergent fibre, uNDF = un-degradable NDF

Table 5.7 Model simplification process, significance values for each individual component of the lmem62 model for time under SARA.

Variable	p value										
	all	-Rum	-BCS	-Prot	-MY	-Lact	-Bfat	-Parity	-BW	-uNDFt	-DIM
Animal											
Parity	0.36	0.45	0.38	0.42	0.32	0.36	**0.39**				
DIM	0.24	0.04	0.04	0.04	0.03	0.02	0.02	0.03	0.04	**0.63**	
BW	0.60	0.43	0.36	0.29	0.31	0.32	0.24	**0.61**			
BCS	0.02	**0.90**									
MY	0.60	0.49	0.60	**0.67**							
BF	0.05	0.46	0.54	0.29	0.45	**0.49**					
Prot	0.71	0.64	**0.74**								
Lact	0.85	0.68	0.55	0.56	**0.47**						
Rum	**0.98**										
PMR											
DM	0.41	0.09	0.12	0.12	0.13	0.08	0.24	0.05	0.05	0.00	0.00
CP	0.01	0.05	0.07	0.06	0.06	0.04	0.04	0.02	0.01	0.00	0.00
NDF	0.06	0.34	0.31	0.33	0.39	0.30	0.27	0.18	0.0.2	0.00	0.00
uNDF	0.51	0.22	0.03	0.03	0.04	0.03	0.02	0.02	0.15		

DIM = days in milk, BW = body weight, BCS = body condition score, MY = milk yield, BF = butterfat, Prot = protein, Lact = lactose, Rum = rumination activity, PMR = partial mixed ration, DM = dry matter, CP = crude protein, NDF = neutral detergent fibre, uNDF = un-degradable NDF

The final and minimal adequate model for lmemean pH was composed by DM, MY and CP (Table 5.6). All factors were statistically significant.

Model lmemeanpH =

mean pH = (5.29-0.0035*DIM+0.0567*CP+0.0444*uNDF)

When rumen pH was defined as time spent under SARA (min/d pH <6.2), the final and minimal adequate model was composed by lmemin62 = BCS, milk lactose content, CP and NDF content of the PMR (Table 5.7). All factors were statistically significant.

Model lmem62 =

Minutes SARA pH<6.2 = (15740.54-28.32*DM-495.64CP-158.78*NDF)

5.3.3.2 Model evaluation

Using data from Trial 4, models lmemeanpH and lmem62 were evaluated for their ability to predict rumen pH values. Table 5.8 shows variables recorded from Trial 4 from 4 animals with rumen pH obtained via rumen pH boluses.

Table 5.8 Summary of variables recorded

Factor	Mean	SD	Range
Mean rumen pH	6.15	0.24	5.41 – 6.58
Time spent under SARA pH<6.2	735	450.4	0 – 1440
Time spent under SARA pH<5.8	208	351.0	0 – 1440
Animal			
Parity	4	1.38	3 – 8
DIM	177	46	94 – 293
BW	680	47.43	572 – 814
BCS (1 – 5)	2	0.5	1.5 – 3.25
Milk yield (kg/d)	33	7	14 – 50
Butter fat (%0	4	0.67	2.74 – 6.36
Protein (%)	3	0.28	2.41 – 4.18
Lactose (%)	4	0.21	3.66 – 4.74
Rumination (min / d)	466	86.88	248 – 638
Feedstuff (PMR)			
DM	44	9.16	28.30 – 56.70
CP	16	1.08	13.60 – 16.80
NDF	37	3.62	33.00 – 43.00
uNDF total	13	2.49	9.90 – 17.70
Sugar	7	1.45	5.30 – 9.70
Starch	15	4.52	5.80 – 19.80

Using this dataset, the model predictions (mean pH and time under SARA) were evaluated. The mean rumen pH predicted by the lmemeanpH model was 5.33 ± 0.11 compared with the observed 5.96 ± 0.14. The LoA (Figure 5.8) showed what appears to be a tendency for values ranging from 5.3 to 5.8 to have differences that increased as pH became more alkaline. The lmemean predicted values were on average 0.63 (95% CI -28 to 1.54) more acidic (lower) than those observed in Trial 4.

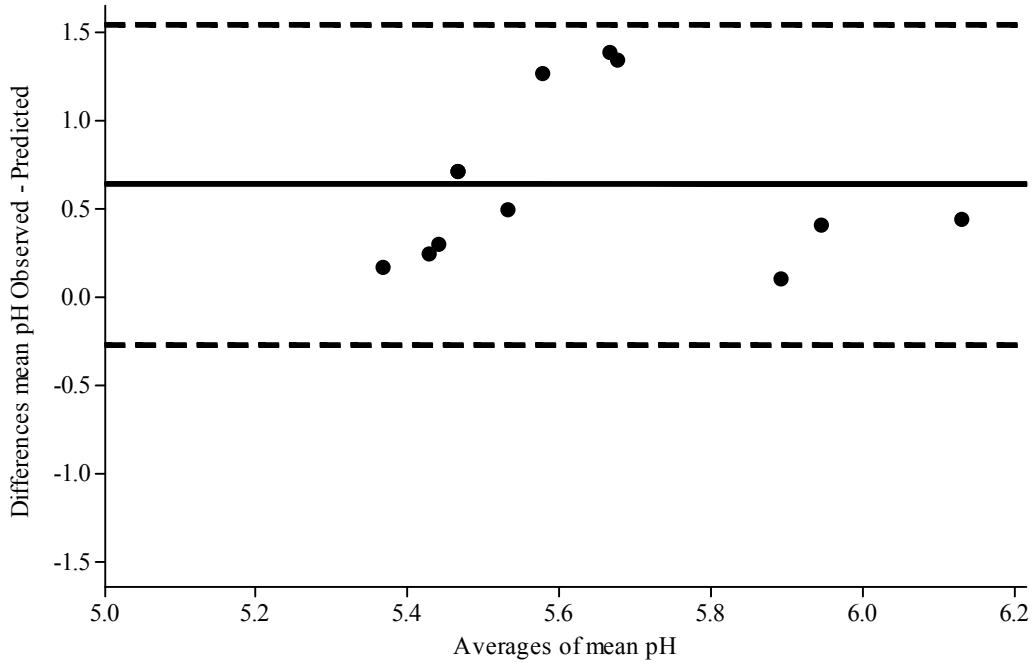

Figure 5.8 The Standard Limits of Agreement method, the plot shows the mean rumen pH obtained with the rumen boluses in Trial 4 and the predictions made with the lmemeanpH model. The solid line shows the mean difference between the two methods (0.63) and the limits of agreement = higher (upper horizontal line 1.54) and lower (lower horizontal line = 0.28).

When analysing the relationship between rumen bolus values and lmemeanpH predicted mean rumen pH values using the mixed effect model, there was no significant relationship (P= 0.41, Figure 5.9).

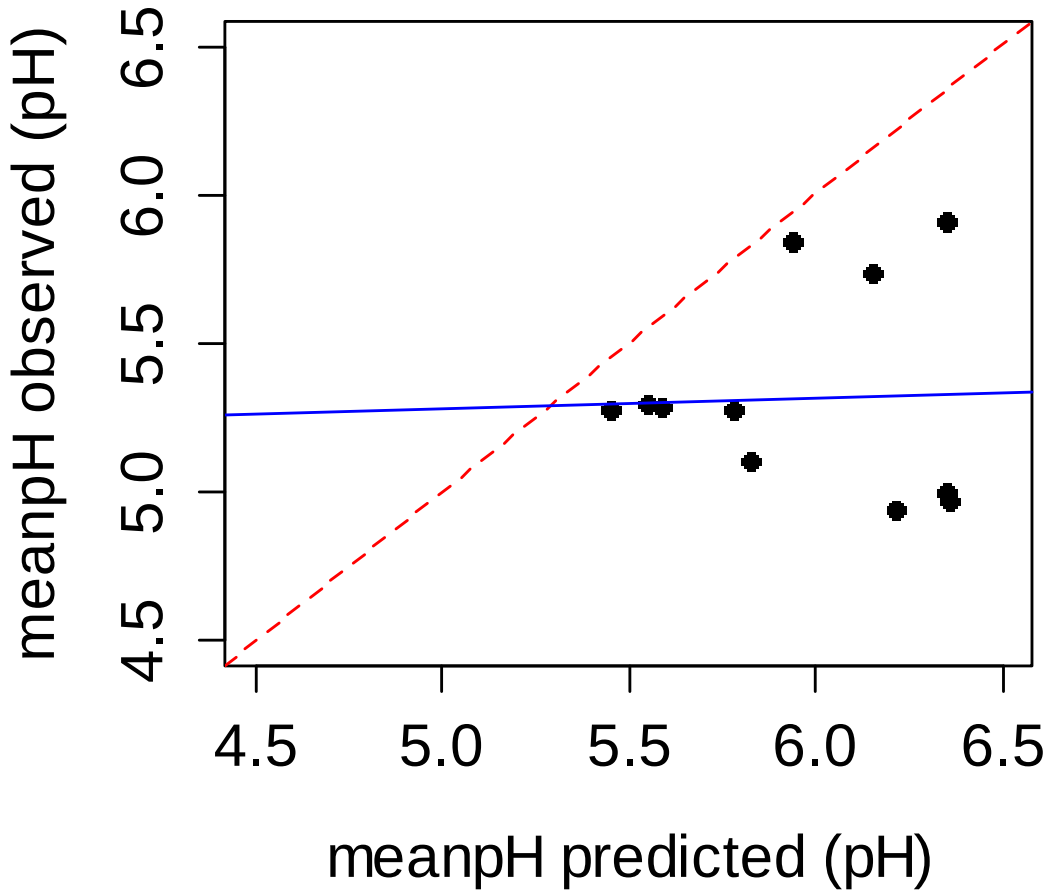

Figure 5.9 Relationship between observed (recorded with rumen boluses) and predicted (model lmemeanpH) mean rumen pH. The broken red line represents the line of equality or perfect agreement, the blue line is the line of best fit between predicted and observed values.

The time spent under SARA (pH < 6.2) was 603 min ± 63 as predicted by the lmem62 and 943 min ± 194 recorded by the rumen boluses. Looking at the LoA (Figure 5.10), the lmemean predicted values of time spent under SARA that were on average 363 minutes (95% CI - 677 to 1403) shorter than those recorded by the rumen boluses. A subtle pattern can be appreciated in which the model tended to under-predict the time spent under SARA, when there were longer periods under pH 6.2 recorded by the rumen pH boluses.

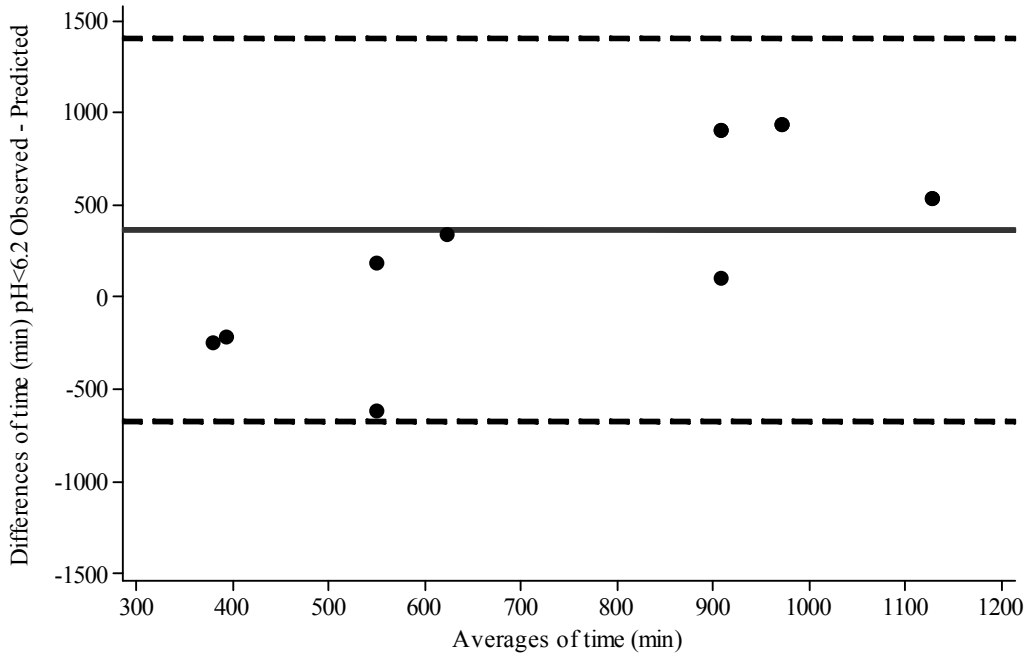

Figure 5.10 The Standard Limits of Agreement method, the plot shows the differences between predicted and observed time spent under SARA. The solid line shows the mean difference between the two methods (363 minutes) and the limits of agreement = higher (upper horizontal dashed line 1403) and lower (lower horizontal dashed line = 677).

When the linear mixed effect model was used to explore the relationship between observed and predicted values for time under the SARA threshold, no relationship was observed (P= 0.92, Figure 5.11).

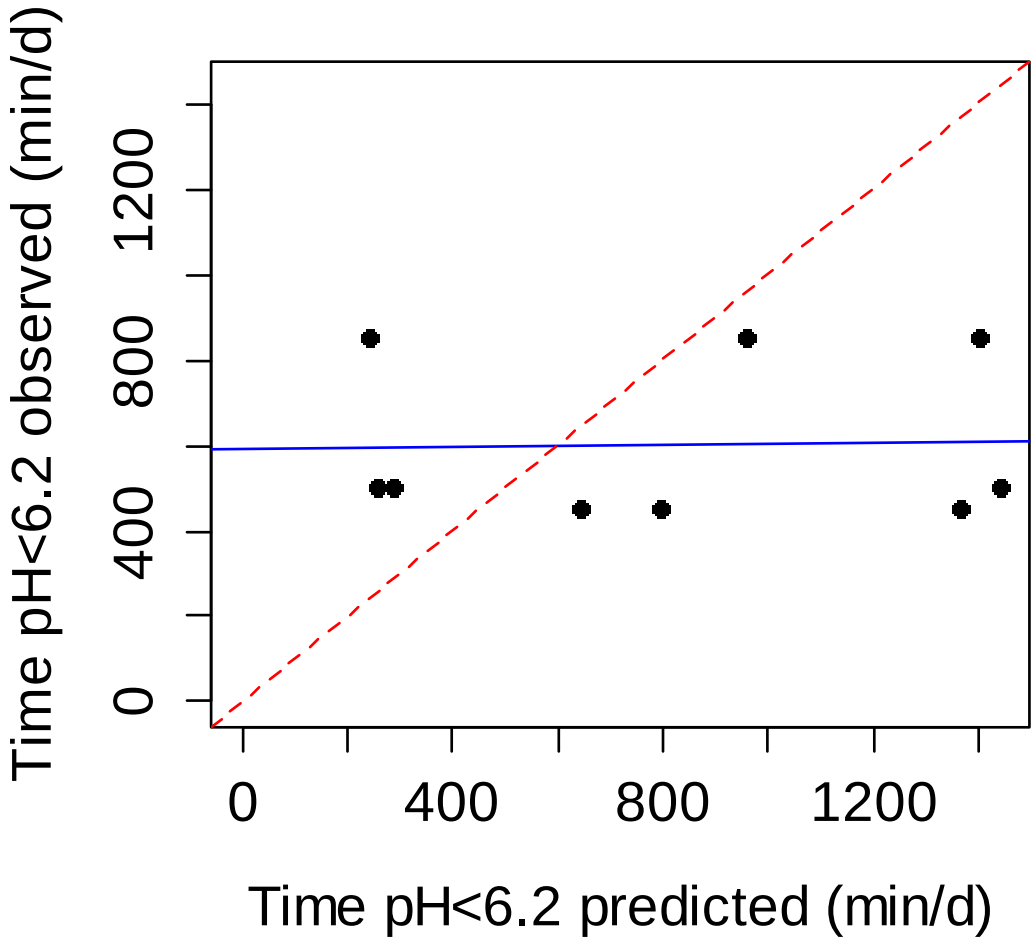

Figure 5.11 Relationship between time spent under SARA (pH<6.2) observed (recorded with rumen boluses) and predicted by the model lmem62. The broken red line represents the line of equality and the blue line is the line of best fit between predicted and observed values.

5.3.4 Discussion

The aim of this work was to develop empirical models to investigate the relationship between rumen pH, animal and feed characteristics measured in the on-farm Trials.

Variables from the animal including MY, BCS, BW and rumination time and from the forages (chemical characteristics) were obtained. In order to define rumen pH, the mean rumen pH per day and the time spent under SARA (min pH<6.2) were used. These values were selected 1) to be able to compare results with previous research (in terms of rumen pH) and 2) to try to describe what is otherwise a very dynamic system.

Mean rumen pH is often used when trying to define or evaluate rumen pH. Rumen pH is predominantly collected using invasive procedures such as via an oro-gastric tube or rumenocentesis. Therefore it is very seldom that more than one rumen pH value is obtained per day, which places constraints when evaluating what is happening within the circadian rumen pH. The information gathered with the rumen boluses helps to understand the fluctuations in daily rumen pH. Hence the definition of rumen pH in terms of a unit that provides information on the pattern of rumen pH values across the day, namely the time rumen pH remains below pH 6.2.

Once the rumen pH measurements were defined, two models were built that contained animal (lmemeanph = MY and for lmemin62 = BCS and milk lactose content) and feedstuff characteristics (lmemeanph = CP and lmemin62 = CP and NDF). The models were not able to explain rumen pH with a linear relationship using the evaluated parameters. As detailed earlier in this Chapter, several empirical models predict rumen pH from dietary characteristics e.g. peNDF which is a known variable related to chewing activity and buffer production, hence influencing rumen pH. However to our knowledge, no other empirical models predict rumen pH from direct animal characteristics i.e. BCS, or outputs other than VFA concentration.

Most modelling exercises have looked at predicting rumen pH from either feed characteristics (Mertens, 1997) or from factors directly related to rumen pH i.e. VFA

concentration (AlZahal et al., 2008; Kolver and de Veth, 2002). However the results obtained were variable.

Similar results to those reported in this Chapter were obtained by Kolver and de Veth (2002). Using Meta-Analysis techniques, the authors explored empirical relationships between rumen pH and animals on pasture-based diets. They found weak relationships (r^2 <0.40) between rumen pH and the recorded variables, and no single dietary variable or group of variables, could be used to make a reliable prediction of rumen pH.

With data obtained from fistulated lactating cows AlZahal et al. (2008) obtained more encouraging results. The authors investigated the relationship between rumen pH and rumen temperature, finding an inverse relationship between rumen pH and temperature. The lowest values of rumen pH recorded in the experimental cows coincided with the some of the highest temperatures recorded at the same time point. The authors concluded that there was scope for rumen temperature to be used as an aid in the diagnosis of rumen acidosis. These were encouraging results, although in this Chapter we did not include rumen temperature as a variable, it could be included in future modelling exercises.

Other researchers have found a strong relationship for all evaluated models between rumen pH, DMI and organic matter (OM) intake, demonstrating that intake level is a principal determinant of mean rumen pH in beef cattle (Sarhan and Beauchemin, 2015). Furthermore the authors concluded that significant relationships between some animal, dietary and ruminal fermentation variables and the model residuals signify that simple empirical models on peNDF or VFA alone do not fully encompass the complexity of factors affecting rumen pH in beef cattle.

The first problem when dealing with the prediction of rumen pH is to find a method of providing a more suitable representation of the highly dynamic system regulating rumen pH. This could be achieved by defining the time spent under certain thresholds of SARA (such measurements were used within this work). Another option could be the creation of an index that measures the time spent under the

threshold ruminal pH by the magnitude of the deviation from this pH (Mackie and Gilchrist, 1979). Although this index might be better related to animal performance than mean rumen pH, variation in rumen pH is more closely related to feeding management practices that affect meal frequency and diet adaptation, than to diet formulation. The effects of feeding management on variation in rumen pH should be considered when choosing the optimal mean rumen pH, which is lower when variation over time is minimised (Allen, 1997). Such models are outwith the scope of this modelling exercise, and out of reach of any other empirical or statistical modelling exercise.

Indeed to be able to predict the behaviour of such a dynamic system that produces circadian rumen pH is a task requiring dynamic mechanistic modelling.

Dynamic mechanistic models can take into account the biology behind rumen pH dynamics. For example they account for the effect of removal of CO_2 gas on bicarbonate buffering in the rumen, saliva flow and composition and variation in microbial cell yield to name but a few key factors involved.

Chapter 6 Results

Rumen pH predictions using a mechanistic dynamic whole cow simulation model

Adapted from: Ambriz-Vilchis V., R.H Fawcett, D.J. Shaw, A.I. Macrae and N.S. Jessop (2015) 8.2. Biopara-Milk: a whole cow simulation model for the prediction of rumen pH. Pages 299 – 306 in: Precision Livestock Farming Applications. Wageningen Academic Publishers (Available in Appendix 2)

6.1 Introduction

Mechanistic mathematical models have been developed that take into account the site of feed digestion, the type of nutrient absorbed and the type of nutrients required for production, thus providing a better prediction of the effect of different feeding strategies on rumen pH dynamics (Dijkstra et al., 2008). The pH in the rumen is a key determinant, and a key product of ruminal digestion. The relationship between ruminal pH and microbial growth means that pH is an important component in nutrient utilization models such as the Cornell Net Carbohydrate and Protein System (CNCPS). The broader biological aims and reliance on more than one data set for parameterization allows mechanistic models wider application than traditional empirical models (Hackmann and Spain, 2010).

For modelling to progress, models should be evaluated and when required modified aiming to improve them (Dumas et al., 2008). Therefore a commercially available mechanistic mathematical, dynamic whole cow simulation model (previously constructed) was evaluated for its predictions of rumen pH – one of many of its outcomes. Using as input data obtained on farm and from laboratory analysis, a detailed description of the animal and the feedstuffs it consumes, the model predicts feed intake, milk yield and rumen pH dynamics. The aim of the present chapter was to evaluate the rumen pH predictions made by the mechanistic model by comparing them with the rumen pH measurements obtained with the rumen boluses in cubicle-housed dairy cows.

6.2 Materials and methods

A mathematical, deterministic, dynamic and mechanistic whole cow simulation model developed by Bioparametrics Ltd. (Edinburgh, Scotland) and commercially available was used. Firstly the model integrates each of the ingredients in a diet or partial mixed ration (PMR) and predicts the daily intake of that particular diet, taking into account the ingredients' characteristics and any constraints imposed by animal size and rumen volume. Secondly the nutrient supply to the animal from obtained daily feed intake is predicted by application of appropriate passage rates of material from the rumen and extent of fermentation within the rumen, small intestine and hindgut. Lastly from the amount and pattern of absorbed nutrients, milk yield and rumen pH dynamics are predicted.

6.2.1 Model description

6.2.1.1 The animal

The model is constructed using the approach first described by Baldwin et al (1987a, b, c) and elsewhere (Hanigan et al., 2006) by following the partition of nutrients through metabolic pathways in the lactating dairy cow. A basic diagrammatic representation of the model is presented in Figure 6.1

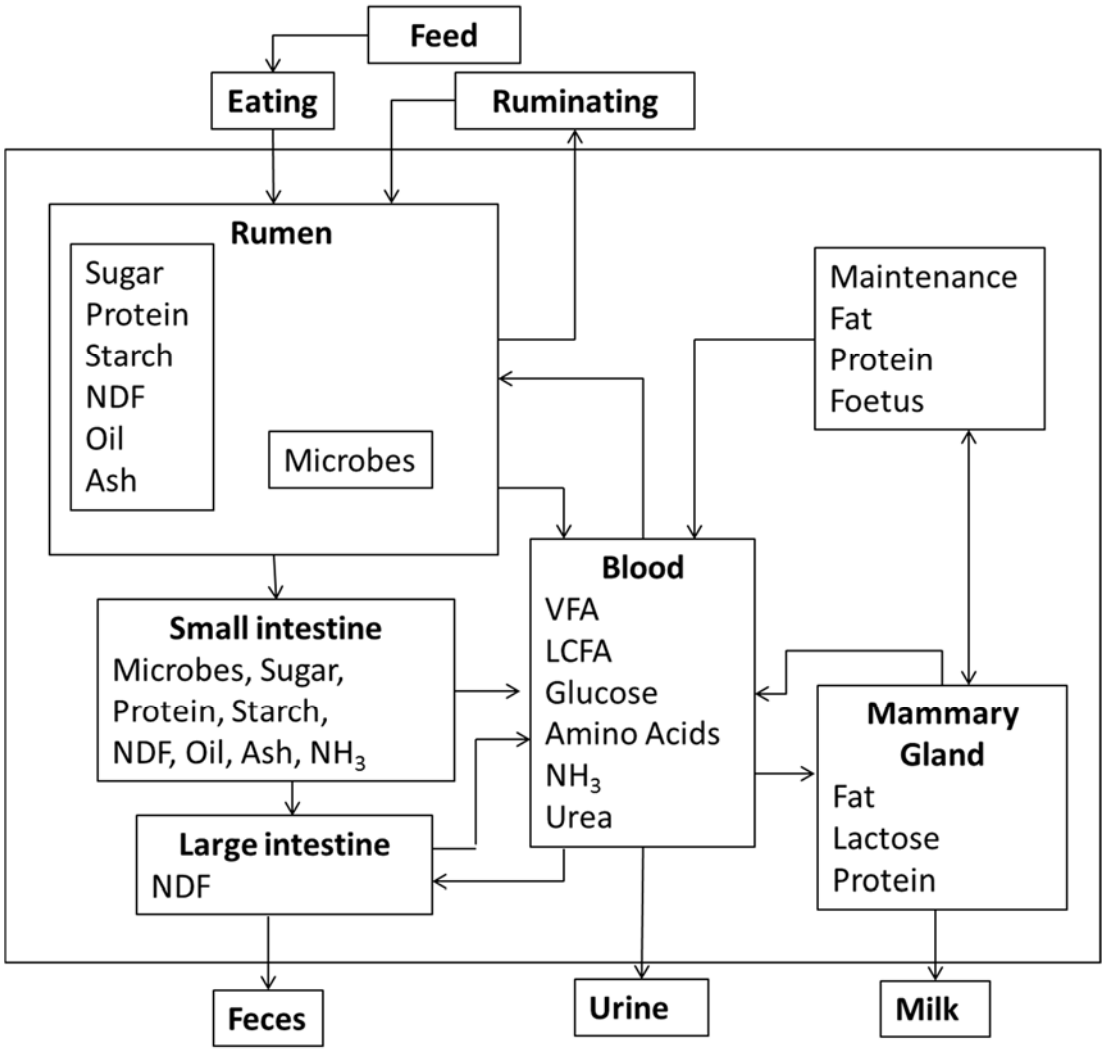

Figure 6.1: Diagram of the basic representation of the animal model. Arrows represent fluxes. Adapted from Hanigan et al. (2006). NDF = Neutral Detergent Fibre, NH3 = Ammonia, LCFA = long chain fatty acids.

To define the animal in the model, details of mature body weight (BW, kg) and body condition score (BCS) on a five point scale (1 – 5) are required. Parity and lactation stage (days in milk, DIM) are used as inputs; genetic potential is expressed as 305 d milk yield, butterfat and milk protein content (both expressed as percentage) are also needed. With these details, the lactation curve (milk yield) can be calculated using the following equation:

$$dY / dt = a\{\exp[-\exp(G_0 - bt)]\}\ [\exp(-ct)]$$

Where dY/d*t* is milk yield per day, *t* is days from calving, *a*, G0, b and c are the coefficients of the Emmans and Fisher (1986) model as described by Friggens et al., (1999).

6.2.1.2 Digestive System.

From knowledge of BW and BCS the size of the rumen-reticulum, omasum, abomasum, small intestine and hindgut are calculated according to Illius and Gordon (1987).

The model encompasses interactions between feedstuffs' degradation characteristics, rumen processes and animal size (Illius and Gordon, 1991). Based on Illius and Gordon (1991), Figure 6.2 depicts the kinetics of nutrient digestion entering the rumen-reticulum model: the form that particles can take (long and short), time for microbes to colonize particles (lag) and fate of particles in the rumen-reticulum (fermentation and passage rates).

In the rumen-reticulum model, the microbiota (bacteria, protozoa, archaea, bacteriophages and fungi) are described as: free microbes (FM) living in the rumen liquor, bound microbes (BM), free lactic acid producers (FLAP), bound lactic acid producers (BLAP) and lactic acid utilizers (LAU) (Figure 6.3). It is assumed that all known species of microbes found in the rumen-reticulum can fit within these five categories. From these populations, it is estimated that lactic acid is produced by FLAP and BLAP. Lactic acid is consumed by LAU or it is passed to the small intestine. Volatile Fatty Acids (VFA) are produced by FM, BM and LAU and are utilised by absorption through the rumen wall or by passage into the small intestine. Ammonia (NH_3) is produced from forage and saliva (urea) and from the metabolism of protein by all the microbial groups. NH_3 can be absorbed or bypassed to the small intestine. NH_3 and Oil are represented as free in the rumen-reticulum model and are available to use by all the microbial groups.

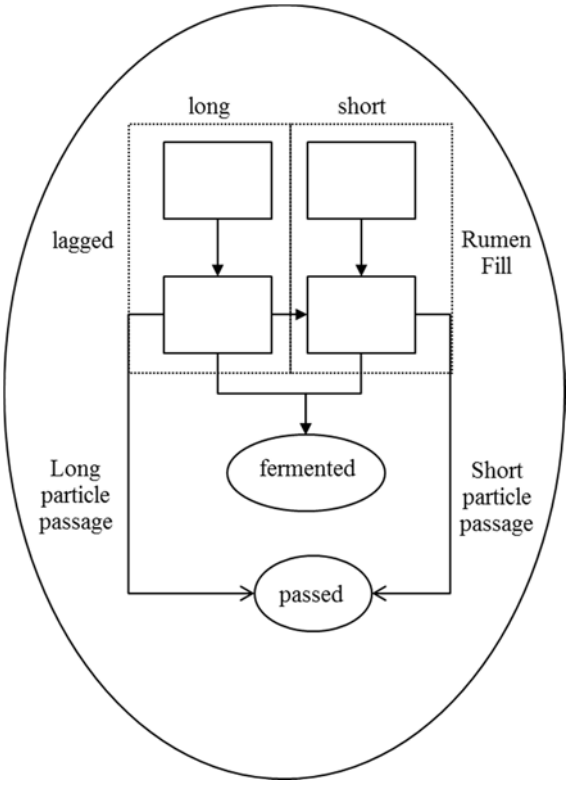

Figure 6.2 Flow diagram of forage digestion in ruminants. Every feed ingredient is described as containing short and / or long particles that can be fermented or passed out of the rumen at different passage rates and after applying times.

6.2.1.3 Feedstuffs

Basic components of the feedstuffs in the model include: Dry Matter (DM), Ash, Oil, Sugar, Starch, NDF, CP and fermentation products (VFA, lactic and NH_3). These are obtained by AOAC International methods (AOAC, 2012). Further analyses which are unique to the model include: degradation parameters of the protein (Figure 6.4a) and the carbohydrates (Figure 6.4b) that are assessed via *in vitro* gas production technique (IVGPT) ((Menke and Steingass H., 1988) with modifications for protein and carbohydrates (Jessop and Herrero, 1996; Palmer, 2006). These analytes are: sugar, other quickly degraded carbohydrates (OQCHO, which are soluble carbohydrates (SCHO) = low molecular weight carbohydrates, pectins and fructans), quickly degradable starch (QS), slowly degradable starch (SS), fermentable NDF (fNDF), quickly degradable CP (QCP, lag = 0 h), slowly degradable CP (SCP, lag > 1 h, as % of DM, lag and fractional rate) (Figure 6.4a and b).

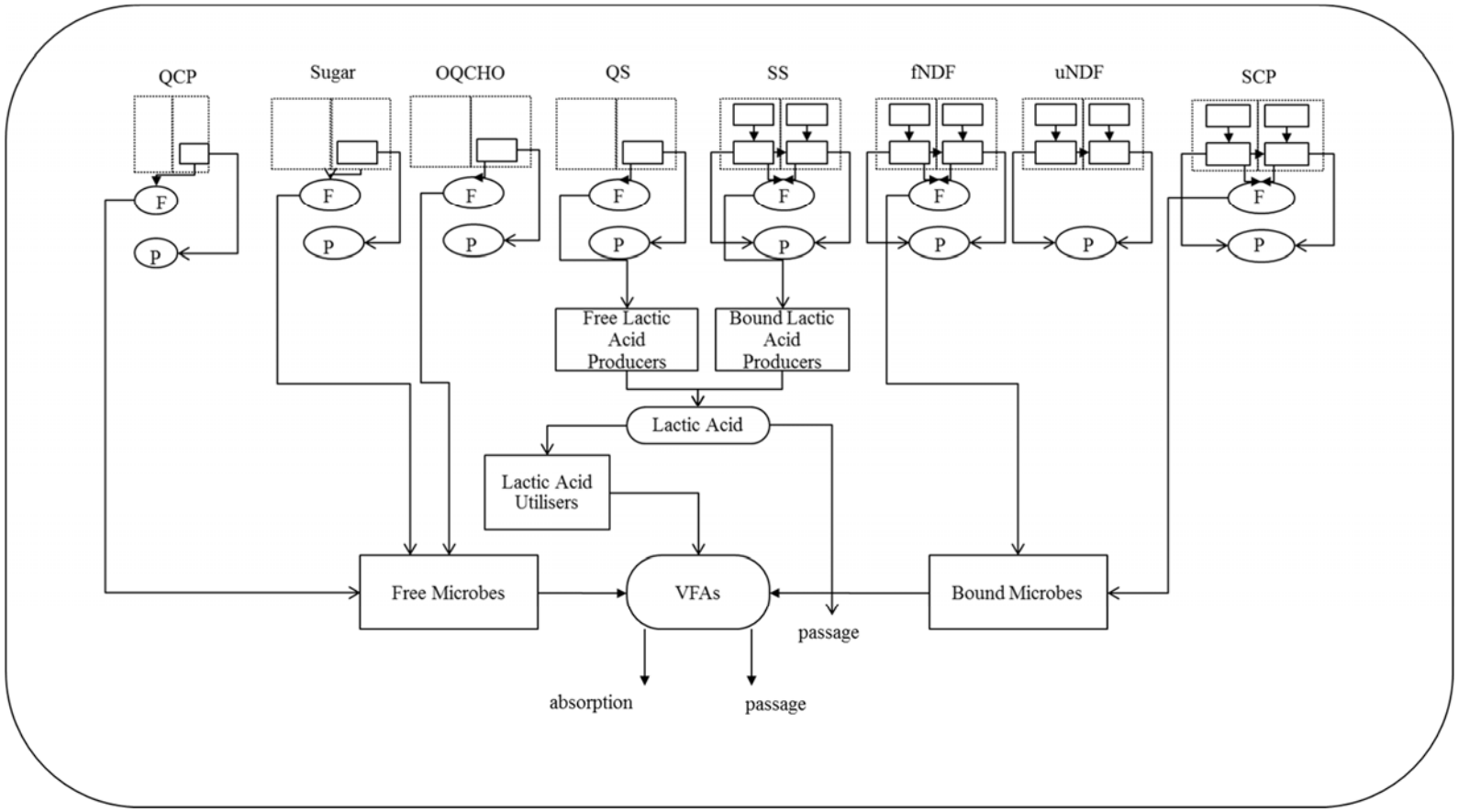

Figure 6.3: Flow diagram of all feed constituents (fractions and fermentation and passage) and the interaction with the rumen microbiota. Availability of all the feed components (Oil, Sugar, OQCHO, QS, SS, fNDF, uNDF, QCP and SCP) passage and fermentation lags and rates are obtained using AOAC analyses and *in vitro* gas production, for each ingredient present in the feeding strategy (partial mixed ration, concentrate, etc.)

The IVGPT provides a better prediction of the *in vivo* digestibility and energetic value of feedstuffs than other techniques, where it is used to represent the fermentation dynamics of the incubated sample (Blummel and Orskov, 1993; Getachew et al., 1998). For each ingredient the amount of sugar, starch and NDF are known, as well as its stoichiometry and the order in which each component is fermented e.g. first sugar, then starch and NDF at the end of the pool (Jessop and Herrero, 1996). With this knowledge, curves to fit the gas production data are produced, and the rate at which the gas is produced along with the lag time are obtained (Figure 6.4a and b).

The model includes a library containing the feeds, forages, minerals, compounds and premixes most commonly used in the UK dairy industry.

6.2.1.4 Feed intake

The predictions of feed intake (FI) result from a dynamic process, based on the concepts of physical (Illius and Gordon, 1991) and metabolic constraints (Emmans, 1997) placed upon the animal to achieve certain intakes, and driven by rumen fill capacity and milk yield potential.

The model assumes that the rumen controls FI. Based on data of BW of the animal, a maximum rumen capacity to hold long and short particles is calculated, and can accommodate a certain amount of the whole analytes described (Oil, Sugar, OQCHO, QS, SS, fNDF, unfermentable NDF (uNDF), QCP and SCP in Figure 6.3) from each ingredient present in the ration (PMR and / or concentrate). Using NDF as an example, the dynamic process involved in the prediction of FI can be described as follows: the NDF from all ingredients in the ration (PMR and concentrate) is obtained, and the maximum rate of intake of NDF in the ration is calculated. Every six minutes, the model calculates how much of the NDF (long and short particles) is lost by fermentation, or bypassed out the rumen (at the corresponding passage rates). The model is based on seven meals per day (Tolkamp et al., 2000).

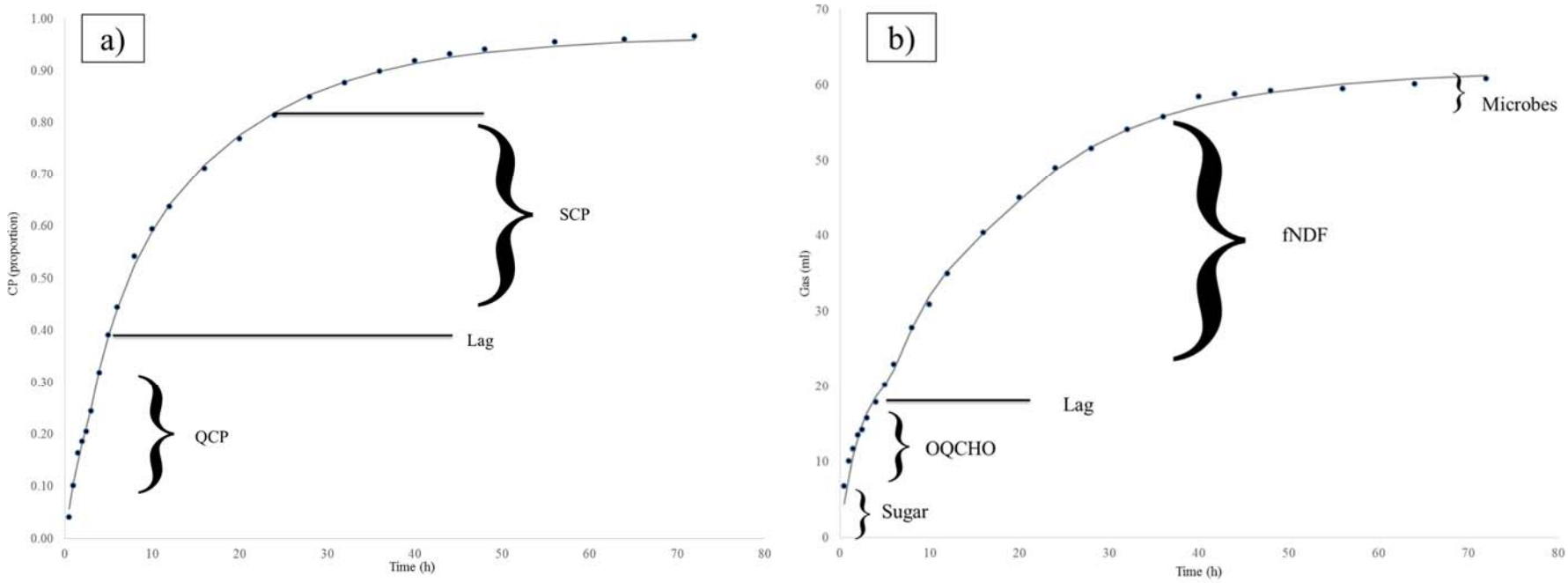

Figure 6.4 Representation of the degradation of the proportion of CP (a) and cumulative gas production profile of the carbohydrates (b) for a grass silage sample. a) quickly degradable QP (QCP, lag = 0 h), slowly degradable CP (SCP, >1 h) and b) sugar (lag = 0 h), other quickly degraded CHO (OQCHO) (soluble carbohydrates = pectins and fructans), fermentable NDF (fNDF) and NDF.

At each time point that the animal eats, according to the restrictions posed by both metabolic and physical constraints determined by diet and genetic potential, intake occurs to increase the amount of food in the rumen-reticulum. Every simulated day, the outputs are checked and if necessary, the rumen fill is adjusted up or down for the next simulated day (it can expand to 1.1 times rumen volume). A steady state is reached by 20 days. The predicted intake is then used to calculate nutrient supply, and from this milk yield, body weight (specifically for protein and lipids) and rumen pH dynamics are obtained.

6.2.1.5 Rumen pH

Following the dynamic prediction of FI, rumen pH is then calculated. Rumen pH predictions are derived from a dynamic process, by continuously estimating the concentration of bicarbonate (HCO_3) in the rumen: i.e. its production and usage (Dijkstra et al., 2012; Kohn and Dunlap, 1998) (Figure 6.5). HCO_3 is produced from: firstly saliva ($NaHCO_3$), secondly by the addition of HCO_3 to the diet (mainly $NaHCO_3$) and lastly by the absorption of VFA through the rumen wall as it results in varying amounts of HCO_3 production from CO_2 (Dijkstra et al., 2012). Saliva is produced at three different rates: resting (production of HCO_3 and urea at low constant rates throughout the day), eating and ruminating (saliva production at high rate for a short period of time and respective HCO_3 levels) (Bailey and Balch, 1961). The amount of saliva produced depends on the animal's size. Baily and Balch (1961) used a 204 kg BW (450 lb) steer: in the present model, the values are scaled accordingly to take account of animal size (weight and BCS). HCO_3 concentration fluctuates depending on it being buffered or removed from the rumen-reticulum. HCO_3 is utilized by its interactions with hydrogen ions, VFA and lactic acid, and it is transported out from the rumen-reticulum at liquid and solid passage rates (Figure 6.5). Lactic acid is produced by FLAP and BLAP; VFA are produced by FM, LAU and BM.

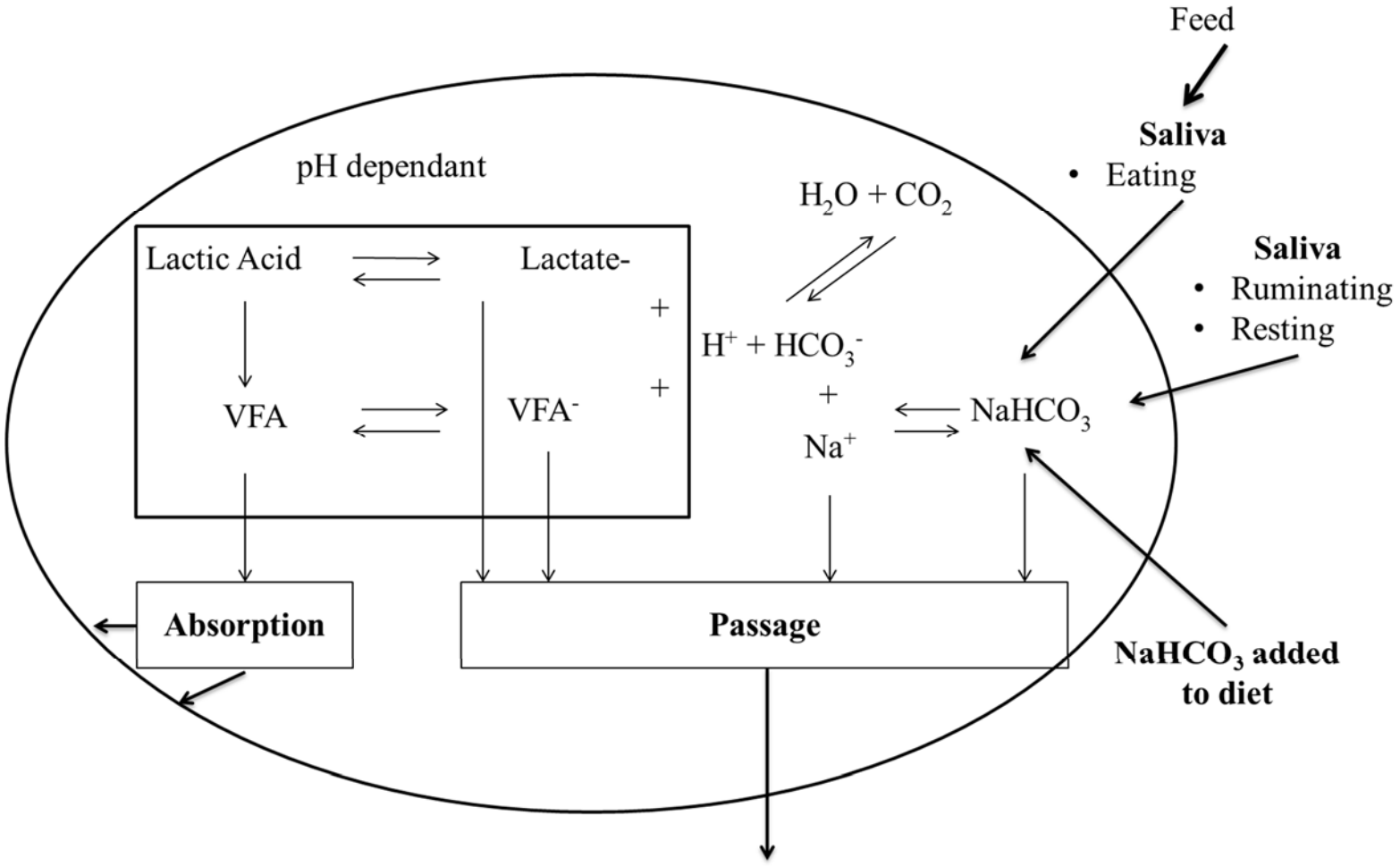

Figure 6.5 Rumen pH system: bicarbonate concentration, production and usage.

All groups of rumen microbes have maintenance energy requirements and these requirements are pH dependent. The relationship between level of maintenance requirements and pH varies according to each pool of microbes, and the effect that pH has on requirements is higher for those three groups that produce VFA than it is for those that produce LA. Every six minutes, the model calculates rumen pH according to Kohn and Dunlap (1998), and after the calculation, the obtained value will be used to modify up or down the energy maintenance requirements for each group of microbes. The energy requirements will in turn affect or influence microbial reproduction and growth, which is a major modifier of microbial ecology i.e. microbial populations and growth rate vary with changes in rumen pH.

From the rates of fermentation and pool sizes, energy and protein are produced from which each group of microbes will be allocated accordingly (Figure 6.3): Free microbes QCP, Sugar and OQCHO; bound microbes from fNDF and SCP. Free LAP from QS, BLAP from SS and LAU from lactic acid. The VFA are produced according to (Reichl and Baldwin, 1975; Reichl and Baldwin, 1976), and Lactic Acid is classed as sugar and fermented accordingly by LAU. As VFA are in free solution they pass out at liquid or solid passage rates, and can also be absorbed at rates according to (Dijkstra et al., 1992).

Every six minutes the model computes the availability of nutrients, requirements and biochemical transaction to then calculate rumen pH using Equation 1 (Kohn and Dunlap, 1998):

$pH = 7.74 + \log([HCO_3]/pCO2)$

HCO_3 = rumen HCO_3 concentration

$pCO_2 = 0.7$

6.2.2 On-farm trials for evaluation of the model: data collection

From Trials 1 and 2 described in detail in Chapter 3, the required data for input to run the whole cow simulation model was obtained.

6.2.2.1 Feed sampling and Analysis

Every week for the duration of Trials 1 and 2, fresh samples of each of the ingredients of the PMR were collected. The samples were sent to a commercial laboratory (Bioparametrics Ltd., Edinburgh, Scotland UK) to be analysed for basic components and fermentation parameters of the carbohydrates and proteins (as described previously) using Near Infrared Spectroscopy (NIRS) calibrated for IVGPT parameters. In addition detailed description of parlour concentrate (ingredients and proportion of inclusion) was obtained.

6.2.2.2 Animal characteristics

Cow weight was recorded using an electronic scale every week during the entire duration of the Trials. Body condition score was recorded weekly by one trained operator to ensure consistency, according to a 1 – 5 scale. Milk yields were automatically recorded at each milking using Alpro 5 computer programme (DeLaval, Cardiff, Wales UK). During the measurement week, composite milk samples per cow were collected at each milking (a.m. and p.m. milking). The samples were then sent to Cattle Information Service laboratory (CIS Laboratory, Herts, England UK) for determination of milk butterfat, protein, lactose.

6.2.2.3 Rumen pH

In both Trials, a rumen bolus (Trial 1 = eCow Ltd., Devon, England UK, and Trial 2 = WellCow Ltd., Roslin, Scotland UK) was orally administered. Prior to deployment, the boluses were calibrated against known standard buffer solutions (Trial 1 = pH 4 and pH 7 Osmotics, Aylsham, England UK and Trial 2 = buffer solutions pH 4 and

pH 7 provided by WellCow Ltd.). Rumen boluses were set to record pH at 15min intervals. The devices have the capability to store the information for up to a month, although data was downloaded every week to prevent losing recorded data.

6.2.2.4 Eating behaviour

Cow behaviour was recorded using video cameras in Trial 1 (described previously in detail in Chapter 2, 3 and 4). From the available videos, cow behaviours were recorded to complete a 24 h period for each cow from a whole week. From the behaviour time budgets, recorded details of the number of meals per day were obtained.

6.2.3 Model Simulation

6.2.3.1 Inputs

Data obtained from the on-farm trials was used to evaluate the rumen pH predictions made by the mechanistic whole cow mathematical model. Details on the individual cow (week of lactation, BCS, milk yield, butterfat (%), protein (%), lactation number) and the consumed feedstuffs (PMR and parlour concentrate) were used to simulate each of the 14 cows in both Trials.

Feeding behaviour (number of meals a day) can be used as input, or as a predetermined parameter in the model (Auto setting). In Trial 1 from the daily time budgets, the meal pattern data was used and entered as input (Input). Alternatively for Trials 1 and 2, it was entered as predetermined by the model on the Auto setting.

6.2.3.2 Output

Rumen pH data per hour for one day (24 h) was obtained for each animal in both Trials.

To further test the assumptions on eating behaviour made in the model and because it is very seldom that data on number of meals a day can be determined in commercial farm conditions, the predictions of rumen pH from Trials 1 and 2 were obtained without knowledge of meal patters using the Auto setting.

6.2.4 Statistical Analyses

Model predictions obtained from each individual animal were used to make comparisons between observed and predicted rumen pH values per hour.

In this study, three different analytical approaches were used to evaluate the models' predictions.

6.2.4.1 Analytical approach 1

To evaluate how well model predictions compare to observed data, a standard approach to model evaluation were used: obtaining measures of deviation. The measures calculated were the Root Mean Square Prediction Error (RMSPE), the Mean bias and the Residual Error. The RMSPE encompasses two terms that relate to systematic problems with models: the mean bias and the error. The RMSPE was calculated as $\sqrt{[\sum(P - O)^2)/n]}$, where O = observed pH values (bolus), P = predicted pH values (model) and n = number of observations. The mean bias was calculated = $\sum(P - O)^2)/n$; it represents the average inaccuracy of the model predictions across all data. The residual error $\sum[RMSPE^2 - (\text{mean bias})^2]$ is the remaining error in model prediction after accounting for the mean bias (Bibby and Toutenburgh, 1977; Kohn et al., 1998).

6.2.4.1 Analytical approach 2

As analytical approach 1 does not explicitly incorporate the repeated measures from each cow, to further explore the agreement between the observed and predicted pH values per hour, a modification of the standard Limits of Agreement (LoA) approach was adopted. This approach was used as it takes into account the multiple observations obtained per individual animal (Bland and Altman, 2007; Bland and Altman, 1986).

6.2.4.2 Analytical approach 3

Finally a mixed-effect model for fitting the residuals (predicted – observed) rumen pH values was also used to evaluate the data. Similar to analytical approach 2, it takes into account the repeated measures obtained from each individual animal (Paterson and Lello, 2003), but also allows fitting of covariates and main effects to the model. The model included which cow that the measurement had come from as the random effect, and to incorporate the effect that time of the day had on rumen pH dynamics a fourth order polynomial for the hour at which each measurement was made was added as a covariate.

The mixed effect model was =

lme = (P-O) ~ hour + I($hour^2$) + I($hour^3$) + I($hour^4$), random = ~1|cowid

P = predicted pH values (model)

O = observed pH values (bolus)

Hour = dime of the day (h)

Cow id = individual cow

All statistical analyses were carried out using R Statistical Environment : the linear mixed –effect analysis was carried out using "nlme" package (version 3.1 – 113 and the modified version of the LoA with repeated measures as modified by Nutter (2008). Statistical significance was taken at $P<0.05$

6.3 Results

6.3.1 Trial 1

Rumen pH data was lost and / or incomplete upon retrieval from five cows, and these incomplete datasets were discarded. Complete data on rumen pH values per hour were obtained using the rumen boluses from nine of the fourteen cows. Daily time budgets were obtained from all of those cows, as well as the relevant information on cow characteristics and feedstuffs consumed required as input to run the model.

Figure 6.6 shows the circadian rumen pH values per cow obtained with the rumen boluses and those predicted by the model.

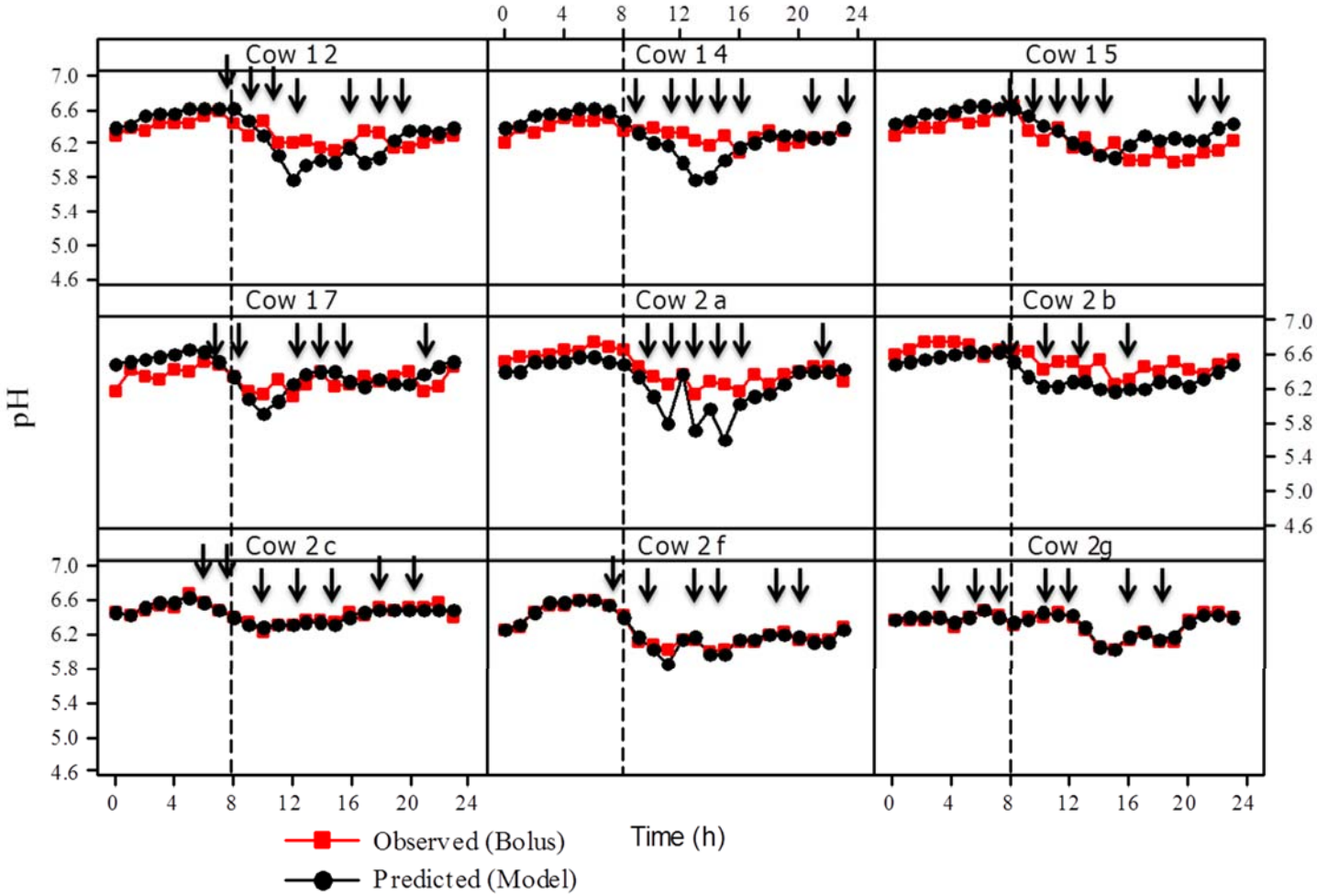

Figure 6.6 Circadian rumen pH dynamics obtained with the model and by rumen pH boluses per cow (Trial 1), the arrows represent each individual meal obtained from analysis of video recordings. The vertical broken lines represent the PMR feed delivery time.

Individual plots (per cow) of the relationship between the two methods in Figure 6.6 shows general good agreement, and the lower panels (Cow 2c, Cow 2f and Cow 2g) show almost perfect match between the data representing the values obtained with both bolus and model predictions. Few discrepancies can be observed on the other panels, the most prominent being the mid central panel (Cow 2a). On these cows the model tends to under-predict (predicts lower rumen pH values) with the model predictions being lower than those reported by the rumen pH boluses. However the pH values obtained by the model are in general agreement with the rumen boluses, and it is capable of predicting the circadian pH fluctuations. The differences between the predictions made by the model and those obtained with the boluses are marginal (less than 0.3 pH units), and present differences on only a few data points (less than 2 h a day).

Statistics of goodness-of-fit (Table 6.1) showed that the predictions of the model with the inclusion of the meal patterns are in general better than those without information of feeding behaviour. There is no tendency for the model to over or under-predict rumen pH (as shown by the LoA plot, Figure 6.7). The knowledge of meal patterns data slightly improved the predictions of rumen pH by the model. However the predictions obtained with the Auto-function of the model resulted in acceptable predictions, very similar RMSPE values were obtained = 0.16, 0.16 and 0.28 for Trial 1 and Trial 2 respectively (Table 6.2). The differences between these values were caused by the under-predicted values reported by the model around 1400, when most of the meals (2 out of 7) are thought to occur.

Table 6.1: Analysis of the relationship between rumen pH observed (rumen boluses) and predicted by the model. The table presents analysis by the modified Limits of Agreement method, mixed effect model and measures of deviation: root mean square prediction error (RMSPE), mean bias and residual error.

	Measures of Deviation			Limits of Agreement method			Mixed effect Polynomial model
	RMSPE	Mean bias	Residual error	Lower limit	Mean	Upper limit	Intercept
Trial 1							
Input	0.156	-0.021	0.024	-0.331	-0.021	0.289	0.090
Auto	0.156	0.036	0.023	-0.268	0.036	0.340	0.091
Trial 2							
Auto	0.276	-0.134	0.058	-0.611	-0.134	0.344	0.101

Further analysis of the residuals from both methods (model vs. bolus) using the LoA analytical approach (Figure 6.7) showed an evenly distributed scatter of measurements with no discernible pattern. The differences between the rumen boluses and the model predictions were evenly distributed across the range. There were no tendencies for the differences between predicted and observed pH values to become larger or smaller as the averages increased. The model tended to slightly under-predict, and the obtained rumen pH values were on average 0.02 (95% C.I. – 0.33 and 0.28, Table 6.1) lower than those recorded with the rumen boluses.

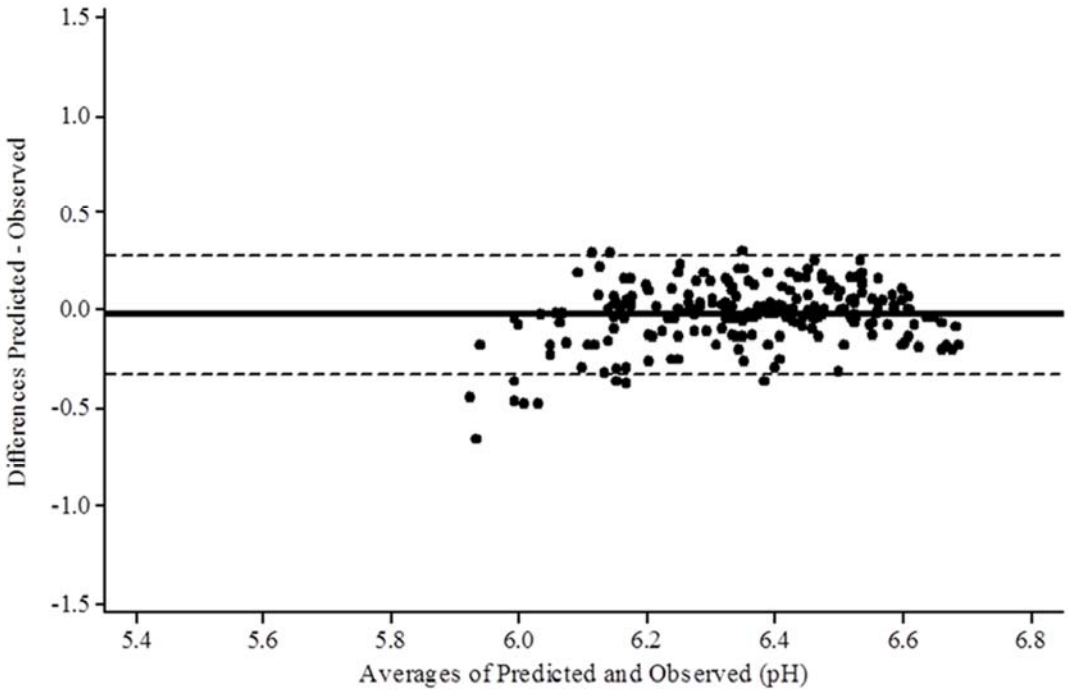

Figure 6.7 The Limits of Agreement analytical approach with multiple observations per individual. The plot shows rumen pH values per hour per cow observed (pH boluses) and predicted with the model (Trial 1). The lines represent the mean difference between the two methods (central solid line – 0.02) and the limits of agreement: higher (top broken line 0.29) and lower (bottom broken line – 0.33).

The regression obtained with the polynomial mixed effect model is presented in Table 6.1 and the residuals are plotted in Figure 6.8. The values follow the trend of

the circadian pH observed on Figure 6.6. The accuracy of the prediction presented by the regression line (red line) obtained with the mixed effect model and that of the mean of the differences (blue line) shows good fit, and no tendencies for the residuals to increase or decrease during the day. The inclusion of the polynomial function takes into account the effect of hour of the day on the rumen pH dynamics.

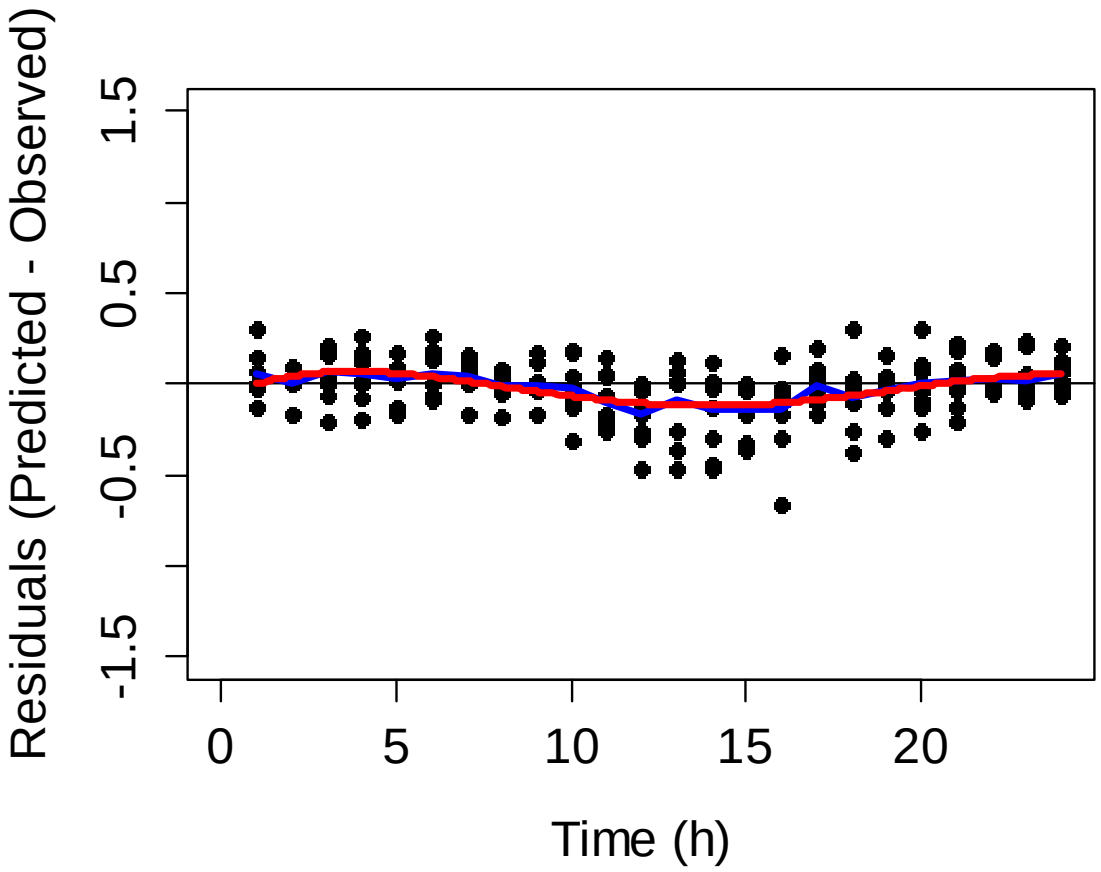

Figure 6.8 Residuals plot of the observed and predicted rumen pH values (Trial 1). Predicted values obtained using eating patterns as input. The solid line represents the equality. The blue line goes through the means and the red line shows the model that best fits the data. Intercept = 0.090.

To further test the model's predictions, model pH data was obtained without data on meal patterns, using instead the predetermined seven meals a day (Auto-mode setting of the simulation model). Figure 6.9 shows the circadian pH per cow obtained with the rumen boluses and those predicted by the model. Individual plots (per cow) of the relationship between the two methods are shown, showing consistently good

agreement observed throughout the day in all the animals. Some of the panels (Cow 1 4 and Cow 2g) show almost perfect match between the data obtained with the two methods. Few discrepancies can be observed on the other panels, however on these cows there is not the tendency for the model to under-predict rumen pH when compared to those values reported by the rumen boluses (as observed previously). Again the model is in general agreement with the rumen boluses, and it is capable of predicting the circadian pH fluctuations.

Figure 6.10 presents the LoA analytical approach, with the plot showing an evenly distributed scatter of measurements with no discernible patterns and a tight scatter of dots. The differences between the rumen boluses and the model predictions were evenly distributed across the range. There were no tendencies for the differences between predicted and observed rumen pH values to become larger or smaller as the averages increased. The model tended to slightly over-predict the rumen pH, and the values were on average 0.04 (95% C.I. – 0.26 and 0.43, Table 6.1) higher than those recorded with the rumen boluses.

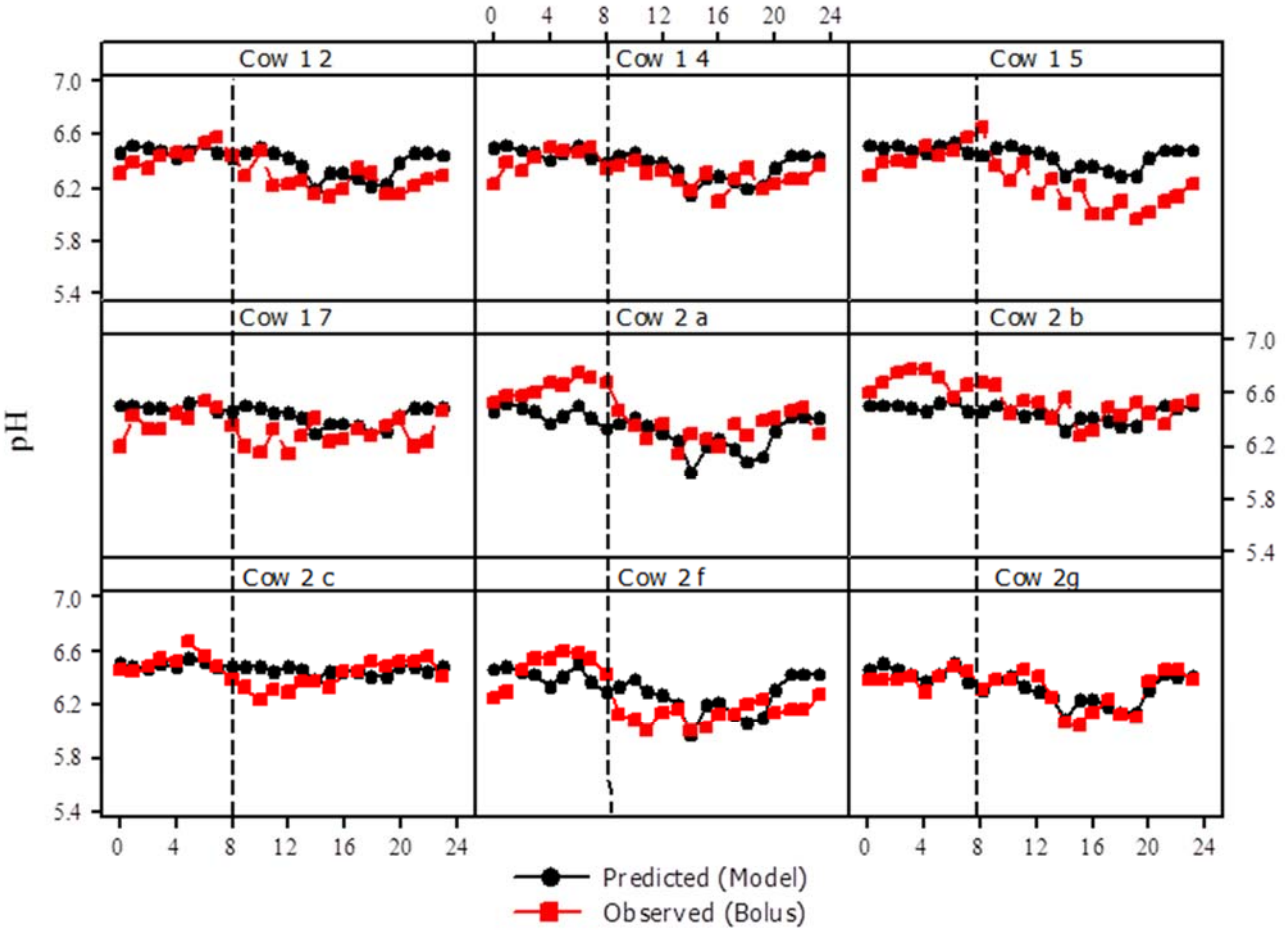

Figure 6.9 Circadian rumen pH dynamics obtained with the model and by the rumen pH boluses per cow. Data was obtained from Trial 1 without eating patterns, using the Auto-mode setting of the model (seven predetermined meals a day). The vertical broken lines represent the PMR feed delivery time.

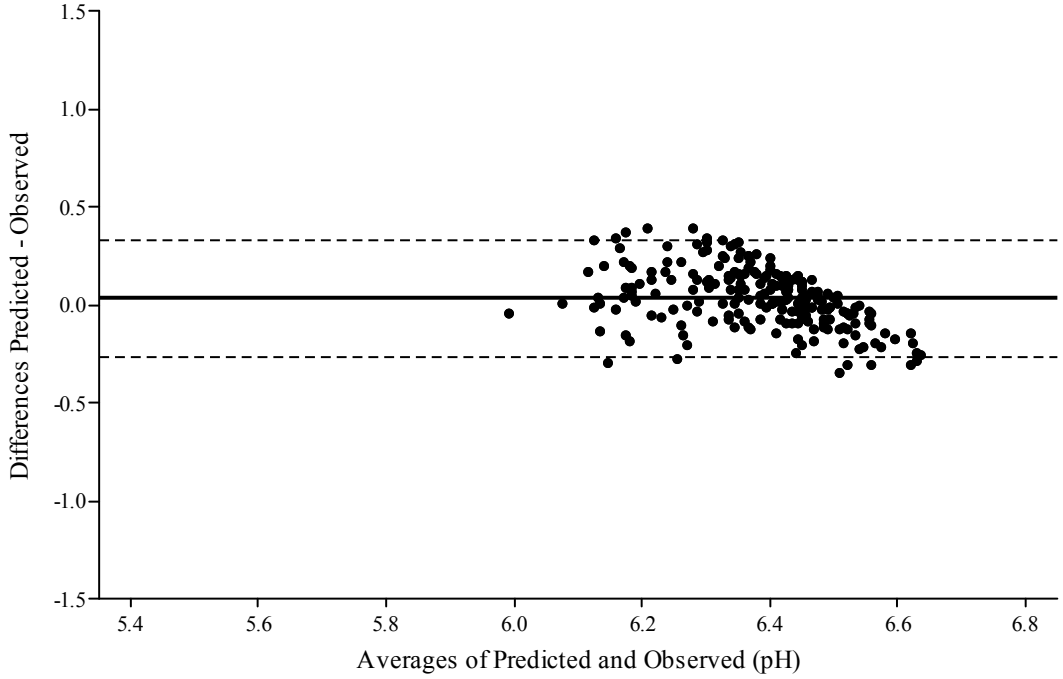

Figure 6.10 The Limits of Agreement analytical approach with multiple observations per individual. The plot shows rumen pH values per hour per cow observed (pH boluses) and predicted with the model (Trial 1). The lines represent the mean difference between the two methods (central solid line – 0.04) and the limits of agreement: higher (top broken line 0.34) and lower (bottom broken line – 0.27).

Figure 6.11 shows the residuals for all the cows during the day. The values follow the trend of the circadian pH observed in Figure 6.9, again the accuracy of the prediction is represented by the regression line (red line) obtained with the mixed-effect model and that of the mean of the differences (blue line). Both lines show good agreement between them, running close to the line of equality, and there are no tendencies for the residuals to increase or decrease during the day.

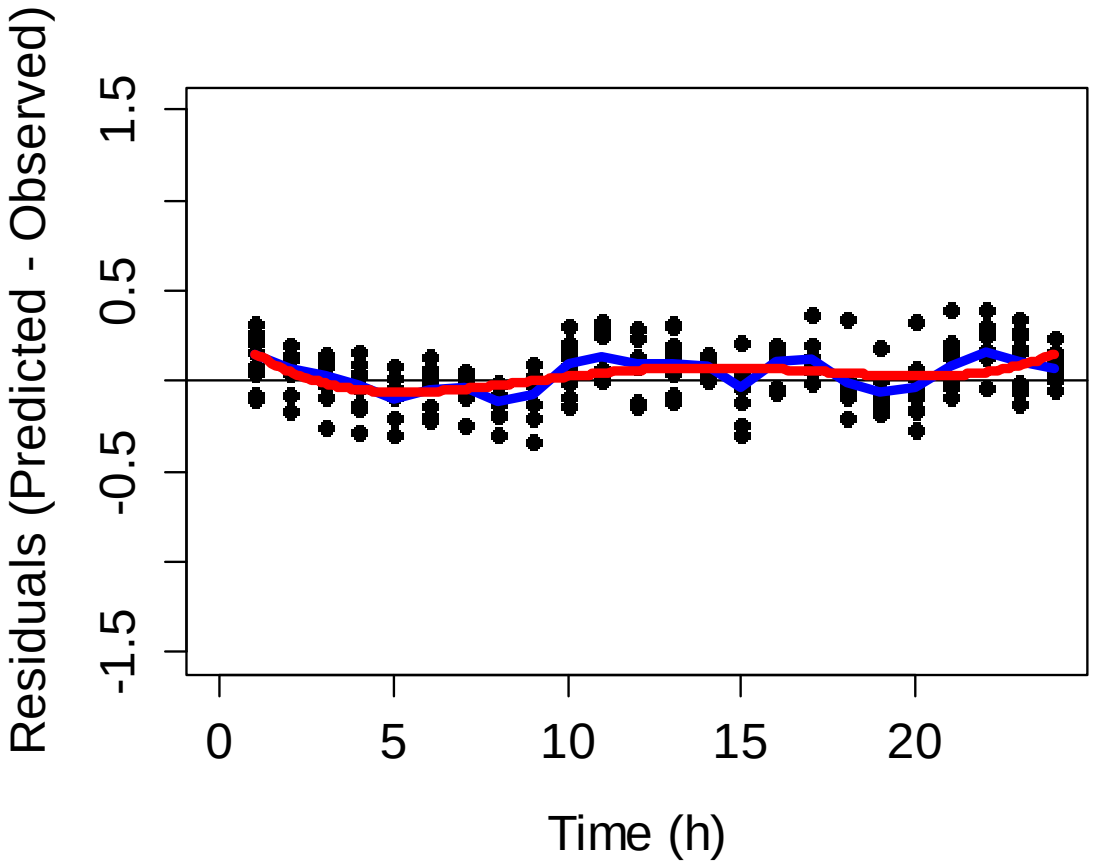

Figure 6.11 Residuals plot of the observed and predicted rumen pH values (Trial 1). Predicted values from Trial 1 were made using the Auto-mode setting of the model (seven predetermined meals a day). The solid line represents the equality. The black line goes through the means and the grey line shows the model that best fits the data. Intercept = 0.091

6.3.2 Trial 2

Reliable rumen pH values were obtained from twelve of the fourteen cows in the Trial. Figure 6.12 shows the circadian pH dynamics per individual cow obtained with the rumen boluses and predicted by the model. Most panels in Figure 6.12 show good agreement between the predictions and the bolus data. However in half of the panels (especially panels in the middle row i.e. cows 143, 145, 146 and 162), the model under-predicted the actual rumen pH value obtained by the boluses. Nevertheless in general the model is in agreement with the rumen pH bolus data, and it is capable of predicting the circadian pH dynamics presented in all the animals as recorded by the rumen pH boluses.

The modified LoA analytical approach plot (Figure 6.13) shows an evenly distributed scatter of measurements, only disrupted by the increased size of differences caused by the under-prediction of the model mentioned before. The model predicted rumen pH values that were on average 0.13 (95% C.I. – 0.61 to 0.34, Table 6.1) lower than those recorded by the rumen boluses, and this under-prediction resulted in the few values that were outside the 95% C.I.

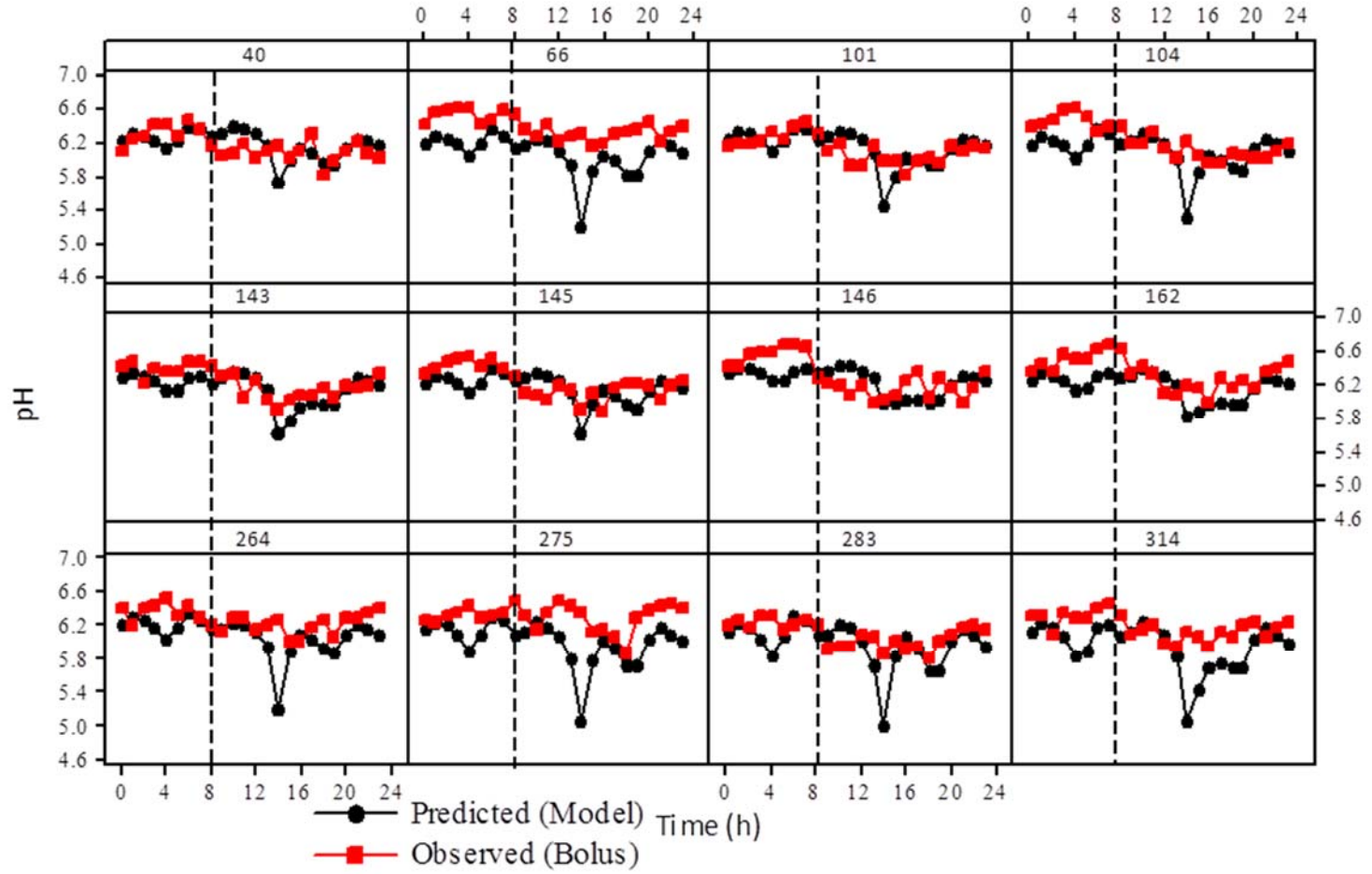

Figure 6.12 Circadian rumen pH dynamics obtained with the model predictions and by rumen pH boluses per cow (Trial 2). The vertical broken lines represent the PMR feed delivery time.

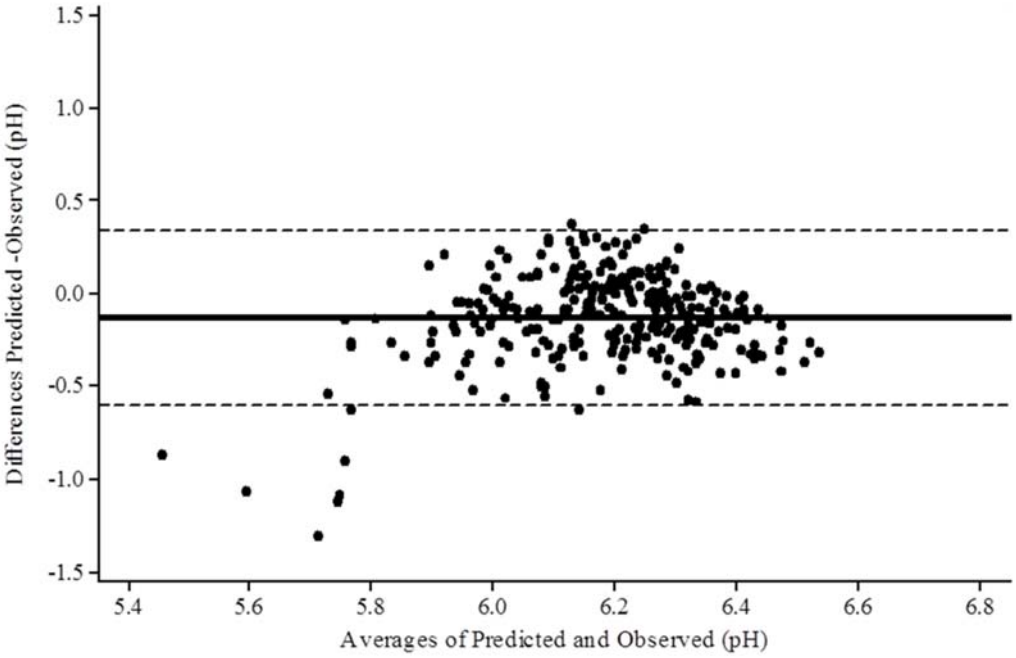

Figure 6.13 The Limits of Agreement analytical approach with multiple observations per individual, the plot shows rumen pH values per hour per cow obtained with the rumen pH boluses and with the model (Trial 2). The lines represent the mean difference between the two methods (central solid line – 0.13) and the limits of agreement: higher (top broken line = 0.34) and lower (bottom broken line – 0.61).

Figure 6.14 shows the residuals plot during 24 h. The values follow the trend of the known circadian pH dynamics, and are tight and close to the line of equality with the noticeable exception of the values around 1400 h when the model tended to under-predict. The lines obtained with the mixed effect model (red line) and the mean (blue line) of the differences do not run parallel and smoothly together as in the previous Figures 6.8 and 6.10.

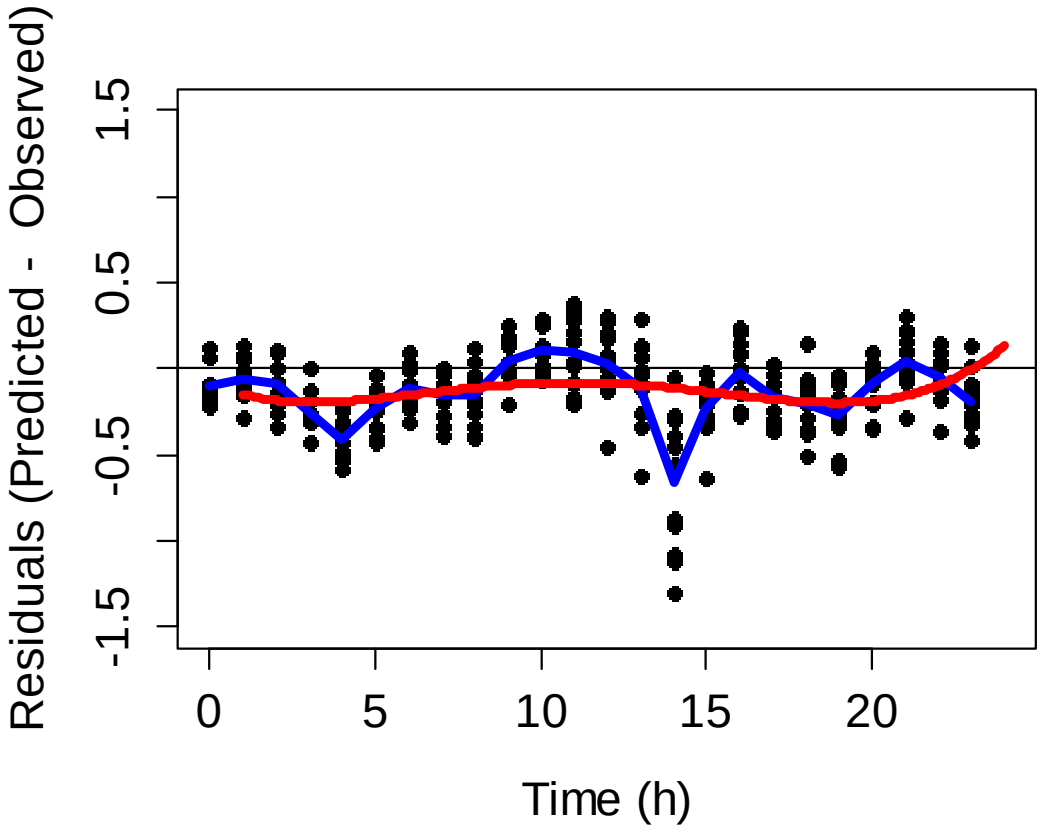

Figure 6.14 Residuals plot of the observed and predicted rumen pH values (Trial 2). Predicted values obtained using the Auto-mode of the model (seven meals a day). The solid line represents the equality. The blue line goes through the mean and the red line shows the model that best fits the data. Intercept = 0.101.

6.4 Discussion

The mechanistic dynamic mathematical model described in this chapter was capable of predicting rumen pH dynamics based on a thorough description of the animal and the feedstuffs it consumes. Describing the dairy cow by the energy and chemical entities it consumes and the interactions throughout its metabolism is the most appropriate and widely used method in other modelling exercises (Hackmann and Spain, 2010; Tylutki et al., 2008).

Using data from NIRS calibrated for proximate analysis and IVGPT it was possible to obtain the required inputs for the model. These data inputs supply the information on lag, fermentation and passage rates required by the model to accurately predict rumen kinetics of the diet, intake and nutrient availability to the animal from which outputs were calculated i.e. rumen pH predictions.

In order to progress with model development, evaluation of the models should be regarded as an essential action. The availability of new technologies (e.g. rumen pH boluses) enables the accurate measurement of pH continuously and for prolonged periods of time. This data enables the evaluation of the rationale behind the model. Predicting rumen pH dynamics involves a number of assumptions: firstly that rumen bicarbonate levels depend on salivary input (variation in saliva production rates between resting, eating and ruminating), passage rate, absorption of VFA and level of bicarbonate in the feed. Secondly, that production of acid depends on the diet and its degradation, microbial metabolism, passage and absorption, and lastly the calculation of resultant bicarbonate levels per hour. Accounting for all of these interlinked processes by means of bicarbonate production and usage is a reliable way to predict rumen pH (Dijkstra et al., 2012; Kohn and Dunlap, 1998).

As shown in Figure 6.5 rumen pH is controlled by many factors, which the model successfully and with small differences (less than 0.5 of pH and for less than two hours per day) was able to integrate to predict the dynamics of rumen pH compared to that obtained from lactating dairy cows. The pH values obtained from the two on-farm Trials showed differences in circadian rumen pH due to animal factors and consumed diets. Although very similar diets were fed, the range of rumen pH values

recorded by the rumen boluses was wide and varied (5.4 – 6.8 pH). Although no extreme low rumen pH values were obtained, these diets are representative of commercial farms in the UK, providing a good scenario to test the model assumptions. Further studies could be carried out to evaluate the models' predictions against other conditions, including SARA.

Observed cow differences in rumen pH are related to multiple and individual factors, such as level of feed intake, eating rate, diet selection, salivation and rumination, inherent ruminal microbial population, previous exposure to acidosis and digesta outflow from the rumen. In Trial 1 the variations in meal patterns were accounted for by using daily time budgets, and using this data as input to the model which resulted in good predictions of the rumen pH values. However when tested using unknown meal patterns (Auto set-up), the assumptions made by the model resulted in similar good predictions in both Trial 1 and 2. Although the number of meals was known in Trial 1, actual feed intake and sorting behaviour were unknown.

It is assumed that animals are trying to achieve a certain daily feed intake to achieve their genetic potential subject to constraints (Ellis et al., 1999), and so are extremely flexible in the organization of their feeding behaviour or number of meals during a day. Therefore the same intakes can be achieved through many different intake patterns (Tolkamp et al., 2000). The under-prediction of rumen pH observed in Trial 2 could be explained by the distribution of meals allocated by the Auto setting of the model, although this setting of seven meals a day is consistent with the literature (Schweitzer et al., 2000). However modification could be made to improve the accuracy of rumen pH predictions. Animals alter their meal patterns and sorting behaviour in response to changes in management conditions e.g. time and frequency of feed delivery. This is also influenced by lactation number and stage of lactation, hierarchy, etc. (DeVries et al., 2011; DeVries and von Keyserlingk, 2008; DeVries et al., 2003; DeVries et al., 2005; DeVries and von Keyserlingk, 2005; Hart et al., 2013; Miller-Cushon and DeVries, 2009).

Diurnal meal pattern shows peaks in meal frequency after delivery of fresh feed and after the afternoon milking (DeVries et al., 2003; Tolkamp et al., 2000). Smaller

peaks around morning milking time were observed, and feed intake during the night is very seldom observed (Tolkamp et al., 2000).

To include or account for the way individual cows adjust their feeding behaviour to adapt to management, social or physiological factors would be a challenging modelling task and outside the scope of the present thesis. In the model used in this Chapter, when the theoretical model cow eats, it does so at a certain constant rate, and activities such as sorting behaviour which can affect rumen pH are outwith the scope of the model. Feed delivery and milking times are factors with the highest effect on eating behaviour, and are easily obtained in commercial farm settings. This data could be added to the model and test if the predictions of rumen pH circadian dynamics could be improved.

Given the results obtained, it is possible to estimate that the basic model structure is accurate, although further evaluation is required in terms of the rest of the model outputs. In terms of rumen pH predictions, model development could be directed to include more detail in the distribution of meal patterns during the day, and inclusion of more detailed meal patterns related with management events that influence meal distribution i.e. feed delivery and milking.

The development of simulation models capable of addressing the prediction of rumen pH dynamics requires concepts and data on the dynamics of nutrient degradation, microbial fermentation and acid removal from the rumen-reticulum. For the mathematical simulation model to produce accurate rumen pH predictions, the patterns of intake, fermentation and passage rates, and the VFA absorption rate all influence the bicarbonate concentration in the rumen, and must be taken into account. Given accurate description of the animals and the feed consumed, the model used in this Chapter was capable of accurately predicting pH dynamics in dairy cows.

However the model requires further evaluation under more challenging conditions such as predicting rumen pH in extreme values i.e. high concentrate or high forage diets. In addition different physiological and management stages such as heifers,

growing animals, transition period, dry period and peak lactation cows were not assessed as part of the work described in this Chapter, and require further evaluation.

There is also the potential to develop new capabilities for the model, for example by using the model as an evaluation and diagnostic tool for rumen related diseases such as SARA.

Chapter 7 Conclusions

7.1 General Discussion

7.1.1 Assessment of various methods to monitor rumen health in dairy cattle.

These results present the first evaluation of the RC under commercial farm settings in both housed and grazing environments. The rumination time recorded by the observer and the RC was highly correlated in indoor Trials. Although rumination time recorded by the observer and the RC indoors were correlated, variations between individual cows were observed and this should be explored further. Possible malfunction of the RC are not easily detected because there is no standard method to determine if the RC is functioning correctly and that its position on the cow's neck is correct at all times. An alternative to correct and control the position of the tag on the cow's neck could be the use of a halter instead of a collar.

However for Trial 3 in cows outside grazing, the relationship was poor. The results obtained in grazing animals were similar to those reported elsewhere (Elischer et al., 2013), where differences between the 2 measurements were evident and the RC recorded rumination activity where there was none. This could be explained by the fact that positioning of the RC changed due to the extra free movement of the cows outside grazing. Furthermore activities such as licking and self-grooming, drinking and other background noises such as rain and wind (which are considerable in outdoor environments) could have interfered with the recordings made by the RC's microphone. Further research is required to investigate rumination activity in grazing environments, which could potentially be different from housed dairy cows and may be dependent on factors such as sward maturity and characteristics, concentrate and/or PMR supplementation, grazing time and pasture availability (stocking density).

Further work is necessary to elucidate the relationship between the RC output and the measurements obtained with the rumen boluses. Rumination and chewing behaviour will have effects on saliva production, rumen buffering and fermentation patterns. No relationship was found when trying to predict rumen pH from rumination activity (described in Chapter 5). However potential relationships should be explored, as cows can modulate feeding and ruminating behaviour when faced with shortage of grazing time or other management conditions (e.g. milking). A theoretical relationship between rumen pH and rumination rime has been described previously, and looking for a causal relationship between bouts of SARA and reduced rumination activity would be worth investigation further.

Regarding the evaluation of the rumen pH boluses presented in Chapter 5, there were issues related to the experimental work and complexity of sampling procedures which may explain some of the discrepancies seen in rumen pH values. However rumen pH is the result of a highly dynamic interrelated process, and how and where it is measured in the rumen it is likely to be critical. Therefore any further studies will need to eliminate potential extraneous sources of variation in rumen pH measurements related to sampling site. Despite these issues encouraging results with the rumen pH boluses were obtained, with no evidence of drift in the measurements of rumen pH recorded during the three months of the experimental period. The consistent differences between rumen pH measurements observed in Trial 4 would imply problems with the experimental methodology, rather than any variation in the measurements performed by the rumen pH boluses.

The use of such novel technologies could aid as a diagnostic or monitoring system in commercial dairy farms. However for this new technology to be of practical use to the dairy producer, details on causal relationship, applicability, reliability and clear interpretation of the data are required (Rutten et al., 2013). The rumination collars in housed cows could be used to forecast events that are crucial to the dairy enterprise such as calving and oestrus. Furthermore the devices could be used as a diagnostic tool for metabolic diseases with known effects on performance. However further

research is needed to determine the relationship between rumination and diet type, as well as the number of animals required for monitoring purposes, which is likely to be related to the amount of individual animal variation in output values. Specific values or thresholds that will trigger the attention of the manager or person in charge of the data are needed for the technology to be of practical use.

Rumen pH is the result of a highly dynamic process that involves animal characteristics (such production and removal of VFA) as well as, feed characteristics (such as NDF, sugar and starch content). The use of rumen boluses could aid in the construction of a general definition of SARA, instead of the current definition which relies on time points obtained by rumenocentesis. By using circadian pH data obtained with the rumen boluses, the level and extent of SARA could be diagnosed by defining scores on "affected", "susceptible" and "normal" animals. Such scores could also be defined for dairy cows under different feeding systems. Furthermore the detailed information obtained with the rumen boluses could enable further research into the relationship between circadian pH, milk yield and milk characteristics (for example butterfat content, and protein:butterfat ratio).

7.1.2 Effect of yeast supplementation on performance, rumination activity and rumen pH

The three Trials presented in Chapter 3 are novel, in that to our knowledge no other studies had looked at the effect of yeast supplementation on circadian rumen pH and rumination activity in dairy cows under commercial environments. The use of new technologies provides data to further explore the effect of feeding strategies (PMR, grazing) and yeast supplementation on variables that were seldom evaluated in the past, or not to the extent that is now possible.

The results of previous studies in dairy cattle on the use of probiotics have been characterised by conflicting results. Effects of yeast supplementation on performance and rumen pH (as a proxy measure of rumen health) had provided contrasting results with several degrees of effect. Many of the benefits of feeding yeast appear to be

greatest in animals undergoing stress or transition (for example around parturition), and they also appear to make the greatest contribution to improving production in situations where animals are exposed to adverse climates, poor quality diets or other stressors. No statistically significant effects of yeast supplementation were observed on any of the parameters measured in the three Trials described in Chapter 3. These results are in line with previous studies that found marginal or non-statistically significant differences in production, rumination time or rumen pH values.

The lack of effect of yeast supplementation could be due to the low prevalence of SARA in Trials 1 and 3, and the moderate acidotic challenge in Trial 2. It is thought that yeast supplementation has positive effects when cows are under physiological or nutritional stress such as early lactation, or due to higher levels of inclusion of concentrate or high levels of starch content. Productivity gains are likely from either increasing rumen pH or decreasing the diurnal variation in rumen pH by controlling the level and extent of rumen pH dynamics, which is where yeast supplementation may be of benefit.

The use of probiotics in dairy cow nutrition should be further investigated, but their use can only be recommended in situations where proven positive results have been found. Therefore the use of yeast should be avoided as a common regular practice, and the benefits of more efficient methods to improve health and performance (for example proper ration formulation and diet presentation) should be advocated. Furthermore the use of yeast and other feed additives to "correct" problems caused for modern feeding strategies should be analysed and challenged.

Response to yeast supplementation would appear marginal at best, and the present studies reported in Chapter 3 showed no positive effect of yeast supplementation. At the time of writing this dissertation, the cost of yeast supplementation was £0.09 per cow per day. However with a current farmgate milk price of £0.20, any recommendation to the dairy producer should aim to increase feed efficiency and therefore profit. If dairy farmers are feeding high nutrient density diets or diets low in fibre or diets predominantly based on maize silage, then the use of yeast might be worth considering. However for grass silage and wholecrop based diets, feeding

yeast would appear to represent no benefit, and should be an additional expense to avoid.

7.1.3 Evaluation of empirical and mechanistic models for rumen pH predictions

The results obtained with empirical models highlight that rumen pH depends on a myriad of variables that are not accounted for by simple empirical equations, demonstrating the need for further modelling efforts that incorporate nutritional and animal factors to improve the capacity of models to predict rumen pH. Such modelling exercises are beyond the scope of empirical modelling to be able to predict the behaviour of such a dynamic system that produces the circadian rumen pH, hence the use of dynamic mechanistic modelling described in Chapter 6.

The use of an already commercially available model provides the means to test assumptions made from a determined system. Given the results obtained, it is possible to estimate that the basic model structure is accurate in predicting rumen pH, although further evaluation is required in terms of the rest of the model outputs. In terms of rumen pH predictions, model development could be directed to include more detail in the distribution of meal patterns during the day, and inclusion of more detailed meal patterns related with management events that influence meal distribution i.e. feed delivery and milking.

In order to progress with model development, evaluation of the models should be regarded as an essential action. The availability of new technologies such as rumen pH boluses enables the accurate measurement of rumen pH continuously and for prolonged periods of time. This data enables the evaluation of the rationale behind the model. Once evaluated and if successful, the models could be further developed for application within decision support systems, for example in nutritional programmes or automated monitoring systems.

Conclusions and future work

The work described in this Thesis provides further evidence on the use of probiotics as feed supplements for dairy cows, by using new technologies to determine the effects of yeast supplementation on rumen pH and rumination time.

The results presented in this Thesis demonstrate that yeast supplementation does not have any beneficial effect on cow health and performance in herds that do not present significant nutritional challenges to the cow. Furthermore the results showed how variable the responses of individual animals were to challenging situations, such as the addition of highly fermentable concentrates (ground wheat) to the diet, or changes in feeding regimes (PMR to grazing). This variability could explain some of the lack of response observed, and should be further explored. Such issues need to be taken into account when devising feeding strategies or new nutritional interventions to improve health and performance.

Such further work would help elucidate the mechanisms that make individual cows cope differently to acidotic challenge. Such mechanisms may be the result of differences in rumen size, dry mater intake, feed passage rate or absorption capacity of VFA. There are also potential differences in the individual microbiota inhabiting the rumen in different cows, which could account for some of these variations in the ability to handle acidoti challenge.

The work presented provides a framework for the use of rumination collars and rumen pH boluses under commercial farm environments. Reliability of the obtained data was assessed, and protocols for collation and data interpretation were obtained. Future work is required to create thresholds for rumination activity and rumen pH dynamics that aid in the detection and ultimately prevention of disease.

The results obtained using rumen boluses to measure rumen pH will help refine current diagnostic methods for SARA. Current techniques using single time point sampling via rumenocentesis and sampling from one isolated part of the rumen are unlikely to be sufficiently accurate in the diagnosis of SARA. Such diagnostic methods are not reliable measures, or an accurate representation of what it is a highly

dynamic ever changing system. The advent of rumen pH boluses enables the construction of a diagnostic tool that relies on a dynamic interpretation of rumen pH. By using circadian rumen pH data obtained with rumen boluses, the level and extent of SARA could be diagnosed by defining scores on “affected”, “susceptible” and “normal” cows. Furthermore it could help with a more accurate determination of the presence of SARA in UK dairy herds.

The improved accuracy of rumen pH data obtained via boluses could be used to further validate and improve dynamic mechanistic models to help with on-farm nutritional management, to better predict performance and improve feed efficiency, farm management and ultimately profitability.

Further research should explore the relationship of the data provided with the rumen boluses and RC. No clear relationship between rumination activity and rumen pH was observed in this work. The differences in individual animal physiology and their associated rumen microbiota can be further explored by making use of these new technologies for automated recording on farm, which might help distinguish those animals that might be more susceptible to the effects of acidotic challenge.

Such recording devices will facilitate the evaluation of the effect that different feeding strategies or supplementation products may have on rumination time, rumen pH and therefore cow productivity and health. Using animals at different physiological stages would be beneficial to extend the work done in this Thesis using mid/late lactation cows, and the fast changing, highly demanding transition period should be the focus of future trials. The strategies that animals at this critical stage around calving use to cope with the differences between demand and supply of nutrients, and declines in intake should be addressed.

Long-term, the aim should be that the information gained via the use of these novel technologies should be incorporated in to the development and evaluation of models that can be applied at the animal or whole farm level. The effects of different feeding strategies can then be tested and evaluated prior to implementation.

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Appendix 1 Ambriz-Vilchis et al. (2015)

This appendix contains a copy of the research article:

Ambriz-Vilchis V., Jessop N.S, Fawcett R.H., Shaw D.J., and A.I. Macrae. (2015) Comparison of rumination activity measured using rumination collars against direct visual observations and analysis of video recordings of dairy cows in commercial farm environments. Journal of Dairy Science Vol: 98, Issue: 3 Pages 1750 – 1758.

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Comparison of rumination activity measured using rumination collars against direct visual observations and analysis of video recordings of dairy cows in commercial farm environments

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ABSTRACT

Automated systems for monitoring the behavior of cows have become increasingly important for management routines and for monitoring health and welfare. In the past few decades, various devices that record rumination have been developed. The aim of the present study was to compare rumination activity measured with a commercially available rumination collar (RC) against that obtained by direct visual observations and analysis of video recordings in commercial dairy cows. Rumination time from video recordings was recorded by a trained observer. To assess observer reliability, data were recorded twice, and the duration of recorded behaviors was very similar and highly correlated between these 2 measurements (mean = 39 ± 4 and 38 ± 4 min/2 h). Measurements of rumination time obtained with RC when compared with analysis of video recordings and direct observations were variable: RC output was significantly positively related to observed rumination activity when dealing with animals housed indoors (trial 1 video recordings: slope = 1.02, 95% CI = 0.92–1.12), and the limits of agreement method (LoA) showed differences (in min per 2-h block) to be within −26.92 lower and 24.27 upper limits. Trial 1 direct observations: slope = 1.08, 95% CI = 0.62–1.55, and the LoA showed differences to be within −28.54 lower and 21.98 upper limits. Trial 2: slope = 0.93, 95% CI = 0.64–1.23, and the LoA showed differences to be within −32.56 lower and 19.84 upper limits. However, the results were poor when cows were outside grazing grass (trial 3: slope = 0.57, 95% CI = 0.13–1.02, and the LoA showed differences to be within wider limits −51.16 lower and 53.02 upper). Our results suggest that RC can determine rumination activity and are an alternative to visual observations when animals are housed indoors. However, they are not an alternative to direct observations with grazing animals on pasture and its use is not advisable until further research and validation are carried out.

Key words: dairy cow, rumination activity, validation, video recording, direct observation

INTRODUCTION

Ruminants occupy an advantageous niche in the animal kingdom. Due to their digestive adaptations, ruminants are capable of converting fibrous, cellulose-rich plant material to energy sources (Van Wieren, 1996). These fibrous materials are first subject to pregastric fermentation, second regurgitated at frequent intervals, rechewed, and finally swallowed back for further degradation.

Rumination reduces the particle size of feedstuffs for rumen degradation, and initiates the process of extracting soluble contents from the feed (Van Soest, 1994). Furthermore, by stimulating saliva production, rumination aids in maintaining correct rumen function by keeping rumen pH within a suitable range for microbial cellulolytic activity (Beauchemin et al., 1989). A combination of factors influences rumination, including nutritional factors, physical and chemical characteristics of the food material, environmental stressors, and day length. For example, rations with fibrous feeds increase chewing activity, whereas high concentrate rations reduce rumination, which could lead to rumen acidosis.

Rumination has a significant effect on intake and forage utilization, which directly correlates to performance, health, and welfare. Therefore, it has been proposed that rumination activity could be used as an indicator of animal health and welfare (Weary et al., 2009). Changes in rumination time may be used as a proxy measure of illness or changes in health status (i.e., if detected, subtle changes in rumination activity could help in the detection of subclinical diseases before they progress and become a clinically apparent concern). To

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further investigate this possibility, accurate and precise methods to measure rumination time are required.

Visual observation is the standard and more reliable method to measure rumination. This can be done either through direct observations or by analysis of video recordings; however, it presents some disadvantages (e.g., requires trained personnel and the number of animals that can be observed at a time is limited). Analysis of video recordings, on the other hand, allows observation of groups of animals and can be performed away from the study site. Video observation also has limitations because it requires trained personal and relies on expensive infrastructure.

To overcome the difficulties posed by monitoring and recording behavior, automated equipment to record feeding behavior (eating, ruminating, or both) have been developed. These devices can measure rumination by means of analyzing jaw movements (Beauchemin et al., 1989; Rutter et al., 1997; Kononoff et al., 2002; Umemura et al., 2009; Braun et al., 2013) or recording sounds of mastication (Laca and WallisDeVries, 2000; Schirmann et al., 2009; Clapham et al., 2011; Elischer et al., 2013; Goldhawk et al., 2013; Navon et al., 2013). Some of these devices have been evaluated in different experimental conditions and with variable results ($P < 0.05$; r = 0.41 to 0.96 and $R^2 = 0.86$ to 0.93).

Automatic recording systems present advantages over visual observations; however, these devices need to be tested and validated to ensure that the obtained data are reliable and accurate. In the past few years the rumination collar (**RC**; SCR Engineers, Netanya, Israel) has frequently been used in the literature (Adin et al., 2009; Gregorini et al., 2012; Soriani et al., 2012; Schirmann et al., 2013; Hart et al., 2013). The RC enables the recording of rumination time from sounds recorded by a microphone with a neck collar, which is positioned to hold the RC microphone on the left side of the cow's neck. The characteristic sounds of regurgitation and rumination are recorded, digitally stored, processed, and then data presented as rumination time either min/2 h or min/d (Bar and Solomon, 2010). Previous studies have evaluated the RC under experimental conditions (i.e., cows confined in individual pens that are not representative of group housing in farm commercial conditions) and cannot be extrapolated to different environments (Schirmann et al., 2009; Burfeind et al., 2011). When the RC were evaluated on other environments (under on-farm conditions), evaluation was either not performed against known rumination behavior (Byskov et al., 2014), or the evaluation showed the RC performance to be very poor and inconsistent (Elischer et al., 2013; Goldhawk et al., 2013). Furthermore, these previous evaluations of the RC did not use statistical analyses that took into account the repeated measures performed on individual cows.

Although the performance or output of the RC has been under scrutiny in the past years, the consensus seems to be that further evaluation and validation are needed (Schirmann et al., 2009; Burfeind et al., 2011; Elischer et al., 2013; Goldhawk et al., 2013). Therefore, the aim of the present study was to compare the rumination activity measured with the RC against that obtained from direct observation and by analysis of video recordings in commercial farm environments with both cubicle-housed and grazing dairy cows.

MATERIALS AND METHODS

Animals

Three trials were conducted at the University of Edinburgh at Langhill Farm, Roslin (Midlothian, Scotland, UK) during 2012 and 2013. The farm has a 240-cow Holstein milking herd. All procedures related to animals were approved by the Veterinary Ethical Review Committee (references: trial 1 VERC 2011–88, trial 2 VERC 30/12, and trial 3 VERC11/13) of the Royal (Dick) School of Veterinary Studies of the University of Edinburgh.

Trial 1. January 2012: fourteen multiparous milking cows were selected and balanced for DIM (mean ± SEM 104 ± 12 d) and parity [median lactation number (**L**) = 4]. The cows were then randomly allocated to 2 different groups: group 1 (**G1**: DIM 103 ± 5.0 d, L = 5) and group 2 (**G2**: 105 ± 4.6 d, L = 4), with 7 cows in each group. Each group was housed in contiguous pens that share identical characteristics: area of feed and water troughs, cubicle/stalls with rubber mattresses top-dressed with sawdust 3 times a week.

Cows were offered a partial mixed ration (**PMR**; first cut grass silage 46.2% (fresh weight PMR proportion), whole-crop wheat silage 18.0%, crimped maize 6.7%, dairy meal 24.1%, and molasses 5.1%), with additional concentrate fed to yield in the milking parlor. Water was supplied ad libitum, and the cows were milked twice daily as per standard farm practice.

Trial 2. January 2013: fourteen multiparous milking cows were selected and balanced for DIM (97 ± 4.3 d) and parity (L = 3). The cows were then randomly allocated to 2 different groups: G1 (DIM 96 ± 2.7 d and L = 3) and G2 (DIM 99 ± 9.2 d, L = 4), with 7 cows in each group. Each group was housed in contiguous pens that share identical characteristics: area of feed and water troughs, cubicle/stalls with rubber mattresses top-dressed with sawdust 3 times a week.

Cows were offered a PMR (first cut grass silage 44.9%, wholecrop wheat silage 17.6%, second cut grass

silage 15.6%, dairy meal 18.5%, and molasses 3.4%), with additional concentrate fed to yield in the milking parlor. Water was supplied ad libitum, and the cows were milked twice daily as per standard farm practice.

Trial 3. May 2013: fourteen multiparous milking cows were selected and balanced for DIM (139 ± 4.5 d) and parity (4 ± 0.4 L). The cows were then randomly allocated to 2 different groups: G1 (DIM 140 ± 6.3 d, L = 4) and G2 (DIM 137 ± 6.8 d, L = 4), with 7 cows in each group. Cows were grazing a ryegrass (*Lolium perenne*) sward during the day and night. In addition, when the cows came in for milking in the afternoon, they were offered a buffer PMR ration (first cut grass silage 45.5%, wholecrop wheat silage 35.4%, Langhill dairy meal 18.9%, and calcined magnesite 0.3%). Additional concentrate was fed to yield in the milking parlor. Water was supplied ad libitum, and the cows were milked twice daily as per standard farm practice. The trial started after a month the cows had been out grazing on pasture.

All Trials. Individual cows were unique to each trial, cows were divided into 2 groups to facilitate management routines (e.g., milking and video recording in trial 1), and to ensure similar parities and DIM between groups of cows in all 3 trials. Cows were milked in a 28/28 herringbone milking parlor (DeLaval, Cardiff, UK) at approximately 0500 and 1500 h. During milking, cows received a minimum of 0.8 kg and a maximum of 6 kg of concentrate a day per cow. All the individuals were clearly identified with a unique number or letter by color spray (Arco Limited, Hull, UK) on either side of the thorax, neck, or both so they were easily viewed and recognized. Cows were given 2 wk to adapt to the diet, facilities, and the RC. All measurements were taken in the third week.

Data Collection

In all trials, a RC (Qwes-HR Lely Ltd., St. Neots, UK) was fitted to each cow to record rumination. A tag reader was located at the exit of the milking parlor so data from the RC were downloaded to and stored, at least twice a day, after each milking. This prevented overwriting of the data because the RC internal memory capacity has only a 22-h storage capacity. The raw data from the RC were then collated. The output presents rumination in minutes per 2-h periods (0200 h, 0400 h, 0600 h or 0100 h, 0300 h, 0500 h, and so on) over a day.

Trial 1. Cow behavior was recorded using 16 video cameras (Panasonic WV BP120, Panasonic, Bracknell, UK) with 1/3″ fixed iris lenses (Panasonic WV-LF4R-5C3AE). The cameras were positioned in key places throughout the shed (fitted to the roof 4.0 and 5.5 m above the ground) so that all cows were viewed and easily identified (by their unique number or letter) at any given time. The area under observation was naturally lit during daylight hours and infrared lighting was used for nighttime recording. The cameras recorded 24 h/d. On an average day, 3 h of cow behavior was missed as the cows left the pens to be milked (around 0500 and 1500 h). Behavioral measurements were analyzed and recorded using The Observer software (Noldus Information Technology, 2004, Wageningen, the Netherlands) by one trained observer using the videotapes recorded during the measuring week. Each cow was recorded continuously for periods of 2 h at a time to complete a full 24-h period per week.

Trials 1, 2, and 3. Cow behavior was recorded by one trained observer using a handheld device (Psion WorkAbout Pro M, Noldus Information Technology). Each cow was recorded continuously for periods of 2 h without interfering with their normal behavior: (a) when cows where housed indoors (trials 1 and 2), the observer was standing in places of the shed where all the behaviors of a specific animal were easily recorded and the observer's presence had no effect on the cow's routine and behaviors (i.e., the animal did not change behavior or moved away from observer); (b) when cows were outside grazing on pasture (trial 3), the observer was standing on the field at a distance (approximately 10 m) where all the behaviors of a specific animal were easily recorded and the observer's presence had no effect on the cow's routine and behaviors (i.e., the animal did not change behavior or moved away from observer).

Behaviors (eating, drinking, idling, and ruminating) were recorded according to the ethogram shown in Table 1. Rumination was defined as the time a cow spends chewing a regurgitated bolus until it swallows it back. Behaviors were recorded continuously (Martin and Bateson, 1994; Mitlöhner et al., 2001) and were defined as being mutually exclusive categories. The 2-h periods recorded were selected so that they matched exactly the period reported by the RC; behaviors were reported in min per 2 h. Behaviors were recorded from available video recordings to complete 24-h period for each cow from a whole week. Direct observations were recorded to match exactly the periods reported by the RC.

Statistical Analysis

Observer Reliability. To test the observer reliability when assessing behaviors from the video recordings, the trained observer scored rumination time twice on 20% of the total observed 2-h periods and the Pearson correlation coefficient between the measurements was calculated.

Relationship Between Rumination Times Obtained with RC and Analysis of Video Recordings.

Table 1. Behavioral ethogram used in trials 1 to 3

Behavior	Definition
Eating	Head over or in the feed trough
Drinking	Head over or in the water trough
Ruminating	Time the cow spends chewing a regurgitated bolus until swallowing it back
Idling	No ruminating, eating or drinking behavior

For trial 1 (video recording analysis), a modification of the standard limits of agreement (**LoA**) methodology was adopted to take account of the multiple observations per individual (Bland and Altman, 1986; Bland and Altman, 2007) and to explore the agreement between the measurements obtained with the RC and analysis of video recordings. When considering the relationship between the 2 variables, a standard linear mixed-effect model was used to resolve the nonindependence associated with the multiple measurements per cow (Paterson and Lello, 2003). In the linear mixed-effect model, which cow that the measurement had come from was entered as the random effect. Additionally, an analysis was made to test whether the slope between RC and analysis of video recordings was different from 1.

Relationship Between Rumination Times Obtained with RC and Direct Observations. For trial 1 (direct observations measurements only), only one measurement was recorded for each individual cow. Therefore, a standard regression analysis and the standard LoA method were used to determine the relationship and agreement between the rumination time obtained by RC and direct observations.

For trials 2 and 3, the standard linear mixed-effect model and modified LoA method with multiple observations per individual were again used. Additionally, an analysis was made to test whether the slope between RC and direct observations was different from 1.

All statistical analysis were carried out using R (R Core Team, 2013) with the linear mixed-effect analysis carried out using the nlme package (version 3.1–113), the standard LoA method using the MethComp package (version 1.22) and a modified version of the LoA with repeated measures as modified by (Nutter, 2008). Statistical significance was taken as $P < 0.05$.

RESULTS

Observer Reliability

Thirty-three 2-h periods [20% of the total 2-h observed periods (164)] were analyzed twice. The twice-observed 2-h periods reported very similar rumination times (mean = 39 ± 4 and 38 ± 4 min/2 h), with a very strong positive correlation between the rumination times obtained from the twice analyzed periods (r = 0.99, $P = 0.001$).

Relationship Between Rumination Times Obtained with RC and Analysis of Video Recordings

In trial 1, behavior was recorded in one-hundred sixty-four 2-h periods from all cows. However, only one hundred thirty-six 2-h periods, when cows were visible at all times, were used for the analysis to determine the relationship between rumination time recorded by the RC and that obtained from analysis of video recordings. The RC recorded a mean rumination time of 45 ± 2 min/2 h that was similar to the mean rumination time obtained by analysis of video recordings 46 ± 2 min/2 h (Table 2). The LoA plot (Figure 1) shows an evenly distributed scatter of measurements with no patterns and there was no clear tendency of the difference between methods to become either larger or smaller as the averages increase. The RC reported rumination times that were on average 1 min (95% CI −24 and 27 min) shorter than those recorded by analysis of videos.

Individual plots of the relationships between the 2 methods showed large variation in the rumination time recorded (R^2 varying from 28.3 to 97.6% with slopes from 0.74 to 1.43, Figure 2). The variability per individual is best exemplified by cows Cd and T1, with poor agreement for cow Cd and data points that match almost entirely with the line of perfect agreement for cow T1.

If the data from all cows were considered, then a significant positive relationship was observed ($P = 0.001$, Figure 3), with the slope very close to 1 (slope = 1.02, Table 2). Excluding cow Cd from the analysis made little difference to this (slope = 1.02). In either case, the slope was not different from 1 ($P = 0.72$)

Relationship Between Rumination Times Obtained with RC and Direct Observations

In trial 1, behavior was recorded in fourteen 2-h periods (one 2-h period per cow). The RC recorded a mean rumination time of 31 ± 5 min/2 h that was similar to the mean rumination time obtained by direct observations 35 ± 6 min/2 h. Using the LoA method, an evenly distributed scatter of measurements with no patterns was obtained. No clear tendency was present for the difference between methods to get either larger or smaller as the averages increase. The RC reported

Table 2. Analysis of the relationship between rumination times (min/2 h) obtained with rumination collar (RC) and analysis of video recordings and direct observations (Obs): regression analysis (trial 1 direct observations vs. RC), limits of agreement method (all trials) and mixed effect model (trial 1 video recordings vs. RC, trial 2 and 3)[1]

			Regression analysis lm(Obs~RC)[2]			Limits of agreement method			Mixed effect model lme(Obs~RC,~1|cowid)		
Trial	n	R^2	Regression equation	SE	P	Lower limit	Mean	Upper limit		SE	P
1 Video vs. RC	136					−26.92	−1.32	24.27	Video = 0.53 + 1.02RC	0.051	<0.001
1 Direct vs. RC	14	0.66	Direct = 0.71 + 1.08RC	0.213	<0.001	−28.54	−3.29	21.98	—	—	
2 Direct vs. RC	28					−32.56	−6.36	19.84	Direct = 8.24 + 0.93RC	0.136	<0.001
3 Direct vs. RC	28					−51.16	0.93	53.02	Direct = 17.66 + 0.57RC	0.207	<0.05

[1]lm = linear model, lme = linear mixed-effects model.
[2]Tilde (~) = operator to separate dependent and independent variables in the model.

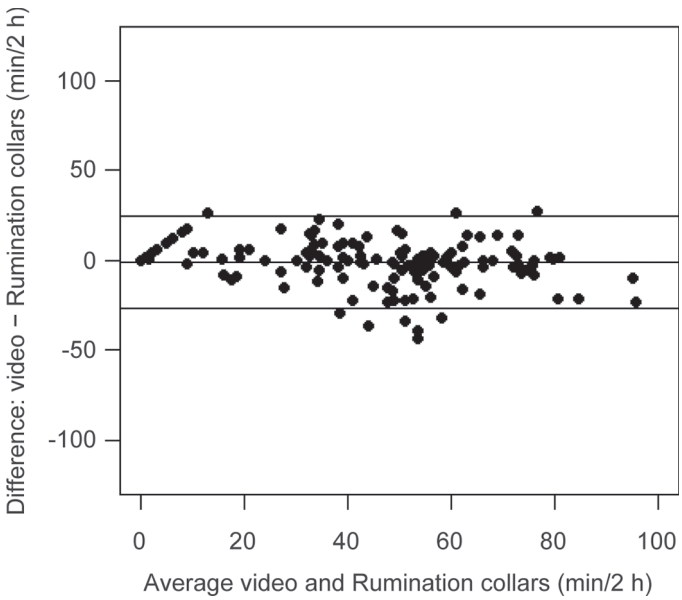

Figure 1. The limits of agreement method with multiple observations per individual. The plot shows rumination time (min/2 h) obtained with the rumination collars and analysis of video recordings in trial 1. One hundred thirty-six 2-h periods were recorded from 14 different cows. The lines represent the mean difference between the 2 methods (central horizontal line, −1 min) and the limits of agreement higher (upper horizontal line, 25 min) and lower (lower horizontal line, −27 min).

rumination times that were, on average, 6 min (95% CI −33 to 20 min) shorter than those recorded by direct observations. The standard regression analysis showed a positive relationship ($P = 0.001$, Figure 4), with the slope very close to 1 (slope = 1.08, Table 2); when testing, the slope was not different from 1 ($P = 0.71$).

In trial 2, behavior was recorded for twenty-eight 2-h periods (two 2-h periods per cow). The RC recorded a mean rumination time of 28 ± 4 min/2 h that was similar to the mean rumination time obtained by direct observations 35 ± 4 min/2 h. The modified LoA method resulted in an evenly distributed scatter of measurements with no patterns or tendencies. The RC reported rumination times that were on average 3 min (95% CI −32 to 20 min) shorter than those recorded by direct observations. As with trial 1, a significant positive relationship was observed ($P < 0.001$, Figure 5), with the slope close to 1 (slope = 0.93, Table 2); the slope was not different from 1 ($P = 0.63$).

In trial 3, behavior was recorded in twenty-eight 2-h periods (two 2-h periods per cow). The RC recorded a mean rumination time of 39 ± 4 min/2 h that was similar to the mean rumination time obtained by direct observations 40 ± 5 min/2 h. As with trials 1 and 2, the modified LoA method showed a scatter of measurements with no patterns and no tendency for the difference between methods to get larger or smaller as the average

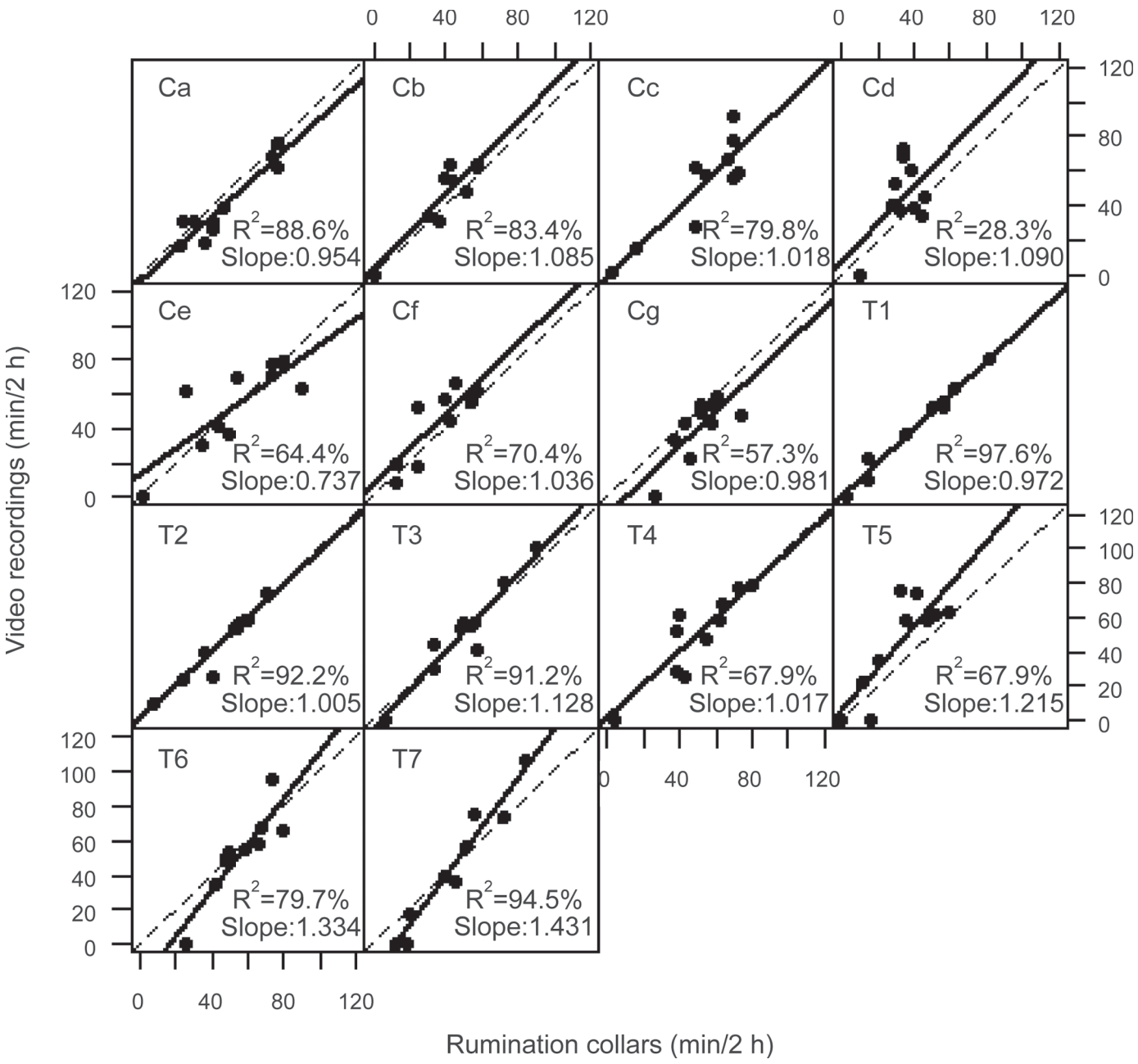

Figure 2. Relationships between rumination time (min/2 h) measured by rumination collars and analysis of video recordings in trial 1. Each panel represents data from one individual cow.

values increased. However, the differences between RC and direct observations were greater than that observed on trials 1 and 2 (with the 95% CI −51 to 53 min, average 1 min longer RC). A significant positive relationship ($P = 0.02$) was observed between visual observation and the RC. In contrast with trials 1 and 2, in trial 3 the slope of this relationship was far from 1 (slope = 0.57, Table 2). However, when tested statistically, the slope was not different from 1 ($P = 0.06$).

DISCUSSION

An accurate and reliable measure of rumination time was obtained by analysis of video recordings with acceptable observer reliability. The observer reliability was similar or even higher than studies in which observers scored rumination time either with direct observations (Schirmann et al., 2009; Goldhawk et al., 2013; Elischer et al., 2013) or from video (Goldhawk et al., 2013).

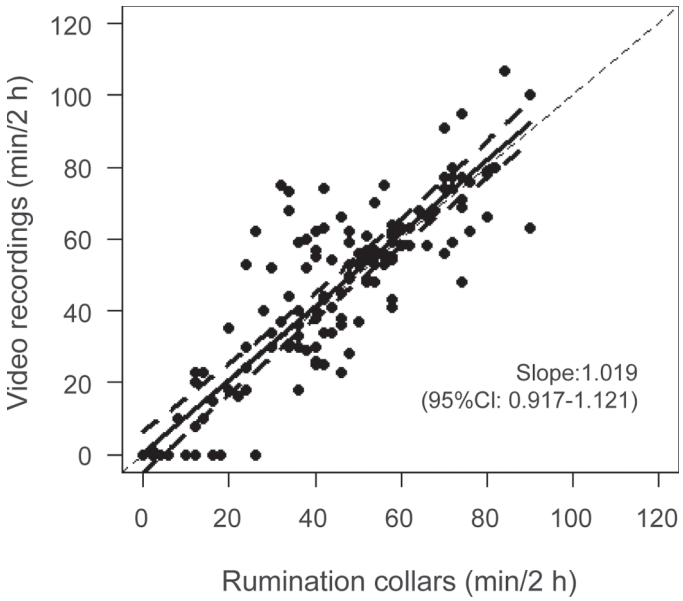

Figure 3. Relationship between rumination time (min/2 h) measured by rumination collars and analysis of video recordings in trial 1. One hundred thirty-six 2-h periods were recorded from 14 cows. The broken line depicts the line of equality on which all points would lie if RC and analysis of video recordings gave exactly the same reading every time. The solid line shows the equation line and the broken thicker lines show the 95% confidence interval.

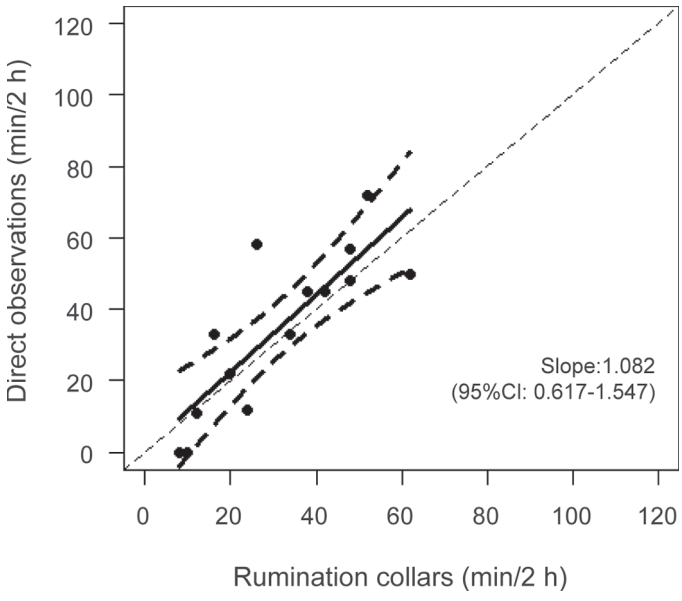

Figure 4. Relationship between rumination time (min/2 h) measured by rumination collars and direct observations in trial 1. Fourteen 2-h periods were recorded from 14 cows. The broken line depicts the line of equality, the solid line shows the equation line, and the broken thicker lines show the 95% confidence interval.

Our results present the first evaluation on the RC under commercial farm settings for both cows housed indoors and for cows grazing grass on pasture, and using a measurement of rumination time by visual observation directly or by analysis of video recordings. It differs from previous evaluations of the RC in that others used controlled settings, by isolating the animals in individual pens to then be observed (Schirmann et al., 2009), or did not use known values of rumination behavior (Byskov et al., 2014). Also, in their previous validation of the RC, Schirmann et al. (2009) and Elischer et al. (2013) reported problems with accurately recording rumination due to the inability of detecting the start and finish of each rumination bout, or due to the fact that the cow's head was not visible to the observer at a distance. In this study, such problems were not an issue. For the analysis of video recordings, only 2-h periods were used when it was possible for the observer to detect start and finish of the rumination event and when the cow was visible; time slots that did not comply with this were eliminated. Three weeks before the start of the recordings by direct observations, cows were accustomed to the presence of the observer. Furthermore, the observer was able to determine start and end of the rumination at all times from a distance far enough as to avoid affecting the cow's natural behavior (i.e., changing current behavior or moving away from the observer).

Although the rumination time recorded by analyses of video recordings and the RC were highly correlated, variations between individual cows were observed. Our results were similar to those obtained on previous

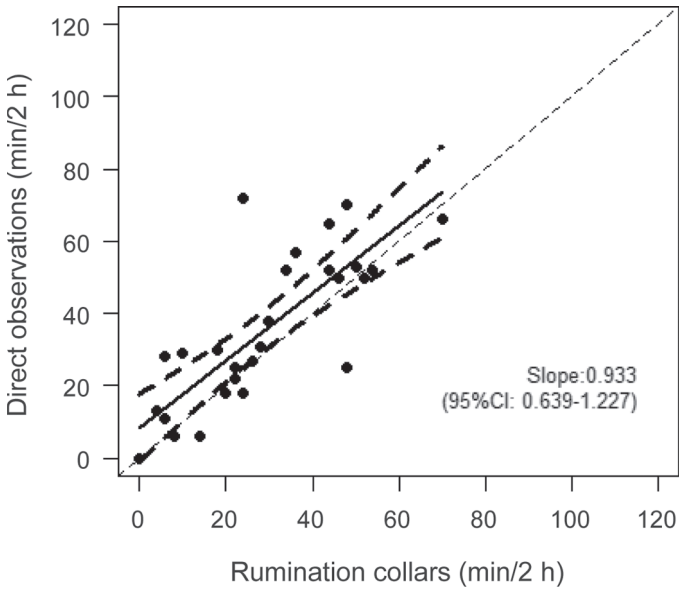

Figure 5. Relationship between rumination time (min/2 h) measured by rumination collars and analysis of video recordings in trial 2. Twenty-eight 2-h periods were recorded from 14 cows. The broken line depicts the line of equality, the solid line shows the equation line, and the broken thicker lines show the confidence interval.

validations of the RC with recorded rumination times varying from 0 to 90 min/2 h (Schirmann et al., 2009; Elischer et al., 2013). The variations on the performance of the RC could be explained by variations between cows: for example, thicker skin that interfered with the microphone, differences in movement that misplaced the RC from the neck, or variation in behavior when ruminating could have affected the RC data (Elischer et al., 2013; Goldhawk et al., 2013).

The rumination time recorded by direct observations and the RC was highly correlated in trials 1 and 2. However, for trial 3 the relationship was poor as the slope was far from 1. The results obtained from the indoor trials were very similar when comparing analysis of video recordings and direct observations. All the trials showed data sets with narrow confidence intervals, a tight scatter of dots, and an equation line with a slope very close to the line of perfect agreement. The results obtained in trial 3 with cows outside grazing showed poor agreement between the RC and the direct observations data set as indicated by wider limits of agreement (−51 to +53 min) shown by the LoA method, wider scatter of dots with wider confidence intervals, and a slope far from 1.

Similarities were found across the 3 trials with previous work performed using cows housed in a pasture based automatic milking system (Elischer et al., 2013), where differences between the 2 measurements of up to 50 min/2 h were recorded and the RC in average recorded, shorter (up to 50 min/2 h) rumination times than visual observations.

In general, although no marked tendency was observed, it is nonetheless noteworthy that in several observations, the RC reported rumination time (1 to 25 min/2 h) when nothing was recorded by the observer (Figures 3, 4, and 6). Similar results have been reported for the RC used with dairy (Elischer et al., 2013) and beef cattle (Goldhawk et al., 2013). This could be explained by malfunctions in one or more of the RC, or by the fact that positioning of the RC changed due to the free movement of the cows around the pen. Furthermore, activities such as licking and self-grooming, drinking, and other background noises (especially when cows on pasture) could have interfered with the recordings made by the RC's microphone. However, no relationship was observed in this study when data from trial 3 were analyzed combining multiple behaviors such as rumination and eating, or rumination and drinking with RC output data. Outdoor farm environments inevitably introduce some level of background noise into a recording, and it can be variable and unpredictable (Navon et al., 2013). This background noise could be the cause of errors in the RC when recording rumination, and cancelling noise technology could be used to improve the RC. Possible malfunctions of the RC are not easily detected because there is no standard method to determine if the RC is functioning correctly and that its position on the cow's head is correct at all times. An alternative to correct and control the correct position of the tag in the cow's neck could be the use of a halter instead of a collar.

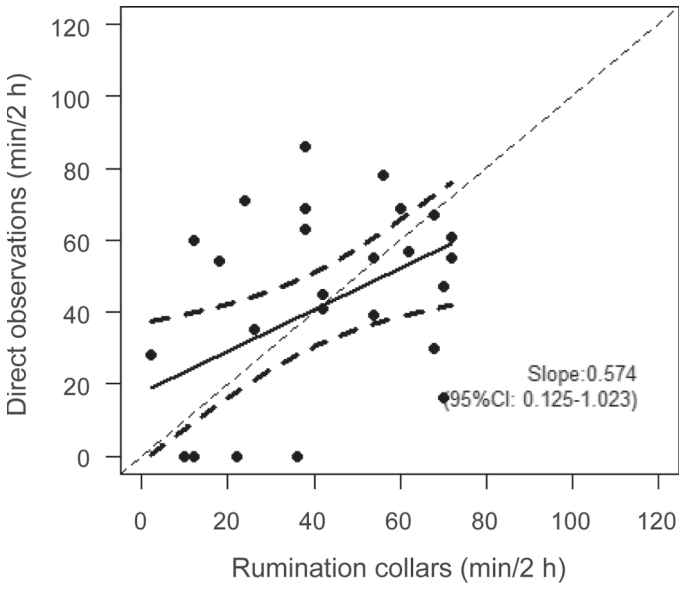

Figure 6. Relationship between rumination time (min/2 h) measured by rumination collars and analysis of video recordings in trial 3. Twenty-eight 2-h periods were recorded from 14 cows. The broken line depicts the line of equality, the solid line shows the equation line, and the broken thicker lines show the 95% confidence interval.

CONCLUSIONS

Measurements of rumination time obtained with RC proved to be acceptable for the conditions of this study when cows were housed inside the shed. However, variations between animals were observed. Our results suggest that the use of the RC in commercial farms can be advised for the determination of rumination activity and are an alternative to visual observations for indoor-housed cows. However, the performance of the RC used with cows on pasture grazing was poor. The use of the RC on cows on pasture should not be advised until further research and validation is carried out. Furthermore, published results that use RC in cows at grass should be taken with caution. Further research is needed to determine a way to ensure that the RC is functioning properly, is placed correctly in the cow's neck at all times, and background noises do not interfere with the RC functioning specially with cows at grazing.

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Appendix 2 Ambriz-Vilchis et al. (2015)

This appendix contains a copy of the book chapter:

Ambriz-Vilchis V., R.H Fawcett, D.J. Shaw, A.I. Macrae and N.S. Jessop. (2015) 8.2. Biopara-Milk: a whole cow simulation model for the prediction of rumen pH. Pages 299 – 306 in: Precision Livestock Farming Applications. Wageningen Academic Publishers

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8.2. Biopara-Milk: a whole cow simulation model for the prediction of rumen pH

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Abstract

Low rumen pH has deleterious effects for the dairy cow: it can alter feed intake, microbial metabolism and feed digestion and cause diarrhoea and laminitis. Amongst other factors rumen pH is affected by diet and so a way to predict the consequences of different feeding regimes on rumen pH would be beneficial. Mathematical modelling is a helpful tool to model the complexity of the rumen and to predict multiple responses of the rumen environment to different diets. Biopara-Milk is a whole cow model, simulating the digestive system and predicting performance and circadian pH dynamics. Intra-ruminal boluses are capable of measuring pH dynamics in non-fistulated animals. The aim of this study was to compare Biopara-Milk pH predictions against those obtained with rumen pH boluses in lactating dairy cows. Fourteen dairy cows were offered a partial mixed ration diet with concentrate fed to yield. Cows were orally administered an intra-ruminal bolus in order to measure rumen pH. Model input data included: detailed information on the feed-stuffs (chemical composition and degradation kinetics) and the animals (bodyweight, condition score, lactation potential, milk composition, week of lactation and lactation number, eating behaviour) and were input into Biopara-Milk. Correlation coefficient (r), concordance correlation coefficient (CCC) and the limits of agreement (LoA) method were performed to determine the relationship between the rumen pH flux obtained with the boluses and the predictions from Biopara-Milk. Average pH values per hour were obtained with both methods and r and CCC for the rumen pH data were acceptable (r=0.93, $P<0.05$ CCC=0.85; n=24,). The LoA showed that disagreements between the two methods were evenly distributed across the range. Estimates obtained with Biopara-Milk were 0.02 (95% C.I.=-0.33 and 0.29) lower than those obtained with the rumen pH boluses. The results showed the capabilities of Biopara-Milk to predict rumen pH dynamics in dairy cows.

Keywords: dairy cow, modelling, rumen pH

Introduction

In the dairy industry, the use of new technologies to measure physiological, behavioural and production parameters can improve management strategies and performance. An example of this is the use of boluses to measure rumen pH. Low rumen pH in dairy cattle can have deleterious effects, such as erratic feed intake, compromised microbial metabolism and feed digestion, and direct negative effects on the health of the dairy cow. Amongst other factors, pH is influenced by feeding regimes. Mathematical modelling is therefore a helpful tool to describe the complexity of the rumen and to predict multiple responses of the rumen to different diets. Biopara-Milk (Bioparametrics Ltd., Edinburgh, UK) is a whole cow model which simulates the ruminant digestive system and, predicts performance and circadian pH dynamics. The aim of the present

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study was to compare Biopara-Milk pH predictions against those obtained with the rumen pH boluses in lactating dairy cows in a commercial farm environment.

Material and methods

Fourteen multiparous dairy cows were selected and balanced for days in milk (DIM) (mean ± standard error of the mean) and parity (median lactation number (L)=4). The cows were randomly allocated to two different groups: Group 1 (G1: DIM 103±5.0, L=5) and Group 2 (G2: 105±4.6, L=4), with seven cows in each group. To facilitate management routines and video recordings, the groups were housed in contiguous pens that shared identical characteristics: area of feed and water troughs, cubicle/stalls with rubber mattresses top-dressed with sawdust three times a week. Cows were offered a partial mixed ration (PMR) consisting of grass silage 46.2% (fresh weight PMR proportion), wholecrop wheat silage 18.0%, crimped maize 6.7%, dairy meal 24.1% and molasses 5.1%, with additional concentrate fed to yield in the milking parlour. Water was supplied *ad libitum*, and the cows were milked twice daily (a.m. and p.m.) as per standard farm practice. To record rumen pH, cows were orally administered an intra-ruminal bolus (eCow Limited, Devon, UK). All individuals were clearly identified with a unique number or letter by colour spray (Arco Limited, Hull, UK) on either the side of the thorax and/or neck so they were easily viewed and recognized. Cows were given two weeks to adapt to the diet and facilities. All measurements were taken in the third week. Cow behaviour was recorded using sixteen video cameras (Panasonic WV BP120, Panasonic, Bracknell, UK) with 1/3' fixed iris lenses (Panasonic WV-LF4R5C3AE, Panasonic). The cameras were positioned throughout the shed so that all cows were viewed and easily identified (by their unique number or letter) at any given time. The area under observation was naturally lit during daylight hours and infrared lighting was used for night time recording. The cameras recorded 24 h per day. On an average day, 3 h of cow behaviour were missed as the cows left the pens to be milked (around 5 a.m. and 3 p.m.). Behavioural measurements were analysed and recorded using The Observer® software (Noldus Information Technology, 2004, Wageningen, the Netherlands) by one trained observer using the video tapes recorded during the measuring week. Behaviours (eating, drinking, idling and ruminating) were recorded according to the ethogram shown in Table 1. Behaviours were recorded continuously (Martin and Bateson, 1994; Mitlohner *et al.*, 2001) and were defined as being mutually exclusive categories, the daily time budget (eating) was used as input for Biopara-Milk model.

The model: Biopara-Milk

This is a whole animal simulation model developed from basic and sound principles of rumen function, microbial growth, feed digestion and passage rates, and animal physiology (taking into account: maintenance, growth, lactation (stage and parity number), pregnancy, and body

Table 1. Behavioural ethogram.

Behaviour	Definition
Eating	head over or in the feed trough
Drinking	head over or in the water trough
Ruminating	time the cow spends chewing a regurgitated bolus until it swallows it back
Idling	no ruminating, eating or drinking behaviour

reserves). At its simplest level, the model uses the ingredients in a diet or partial mixed ration and predicts the daily intake of that diet taking into account any constraints imposed by animal size and rumen volume. The nutrient supply to the animal from the daily feed intake is then predicted by application of appropriate passage rates of material from the rumen (liquid, small and large particles for forages, small and large particles for concentrates) and extent of fermentation within the rumen (each feedstuff has up to seven fermentation rates). Milk yield and/or body weight change (separately for protein and lipid) are then predicted from the amount and pattern of absorbed nutrients. Rumen pH is predicted for a 24 hour period, based on the amounts and pattern of feed consumed and fermentation and passage rates. Rumen pH predictions are derived from a dynamic process by continuously estimating the concentration of bicarbonate in the rumen: i.e. its production and usage (Dijkstra *et al.*, 2012; Kohn and Dunlap, 1998) (Figure 1). Bicarbonate is produced, firstly, from saliva at three different rates: resting, eating and ruminating (Bailey, 1961), secondly by the addition of bicarbonate to the diet, and lastly by the absorption of volatile fatty acids (VFA) through the rumen wall as it results in varying amounts of bicarbonate production from CO_2. The amount of bicarbonate produced depends on the animal's size. The bicarbonate is used as a result of its interactions with hydrogen ions, and from its movements from the rumen at liquid and solid passage rates. Salivation produces bicarbonate and urea, at a low and constant rate from resting and from eating, and at a high rate for a short period of time from rumination.

Biopara-Milk is a simulation model that calculates outputs every six minutes throughout the day. Every simulated day, the outputs are checked and if necessary, the rumen fill is adjusted upwards (there is a maximum) or downwards for the next simulated day. A steady state is reached by 20 days.

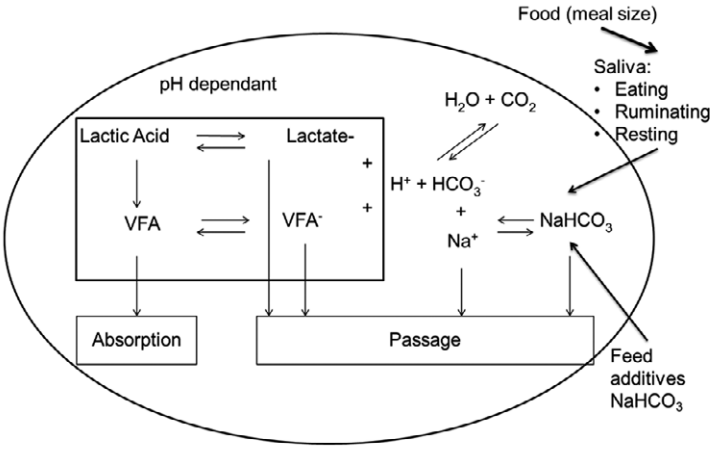

Figure 1. Factors affecting rumen pH: bicarbonate concentration, production and usage.

Model inputs

Animal parameters

Current liveweight (kg), condition score (1-5 scale), lactation potential (305 days yield), milk composition (butter-fat % and protein %), lactation number (heifers, second lactation and third or more lactations) and eating behaviour. Eating behaviour can be entered in five different ways: automatic or Biopara-Milk uses a predetermined meal pattern of eight meals, six meals, four meals or set meal times, i.e. from 3 to 11 meals can be set during a 24-hour period.

Feed-stuffs

Biopara-Milk uses libraries containing a detailed description of all feeds, forages, minerals, compounds and premixes. A detailed description of the feed ingredients is required, including fermentation rates and lags for carbohydrates and protein measured by the *in vitro* gas production technique (Menke and Steingass, 1988). The gas production parameters are routinely predicted by near infrared spectroscopy for most of the forages commonly found in northern temperate climates. The parameters required for the model are: dry matter, ash, oil, sugar, starch, neutral detergent fibre, protein and fermentation products (VFA, lactic and ammonia) obtained by AOAC International methods and degradation parameters for carbohydrates and protein (lag and rates).

Model outputs

Biopara-Milk predicts dry matter intake, milk yield and rumen pH dynamics. Data on the animals and feed characteristics obtained from the feed trial were used to run the Biopara-Milk model. Predictions obtained for each individual animal were used to make comparisons between observed and predicted rumen pH values per hour. A modification of the standard limits of agreement (LoA) methodology was used to take account of the multiple observations per individual (Bland and Altman, 1986, 2007), and to explore the agreement between the predicted and observed pH values. To further assess this relationship and to avoid temporal pseudoreplication due to repeated measurements from the same individual animal, the correlation coefficient (r) and concordance correlation coefficient (CCC) were obtained from pooled data for the means of pH per hour for all individual cows. All statistical analyses were performed using R (R Core Team, 2013), using the modified version of the LoA with repeated measures as modified by (Nutter, 2008) and CCC using the 'Epi.R' package (version 0.9-58). Statistical significance was taken as $P<0.05$.

Results

Reliable pH values per hour were obtained with the intra-rumen boluses from nine of the fourteen cows. Figure 2 shows the circadian pH dynamics per cow obtained with the rumen pH boluses and with Biopara-Milk.

The LoA method (Figure 3) showed an evenly distributed scatter of measurements with no discernible patterns; the disagreements between the two methods were evenly distributed across the range. There were no tendencies for the differences between predicted and observed pH values to become larger or smaller as the averages increased. The pH predictions obtained with Biopara-Milk were on average 0.02 (95% CI -0.33 and 0.29) lower than those recorded with the boluses.

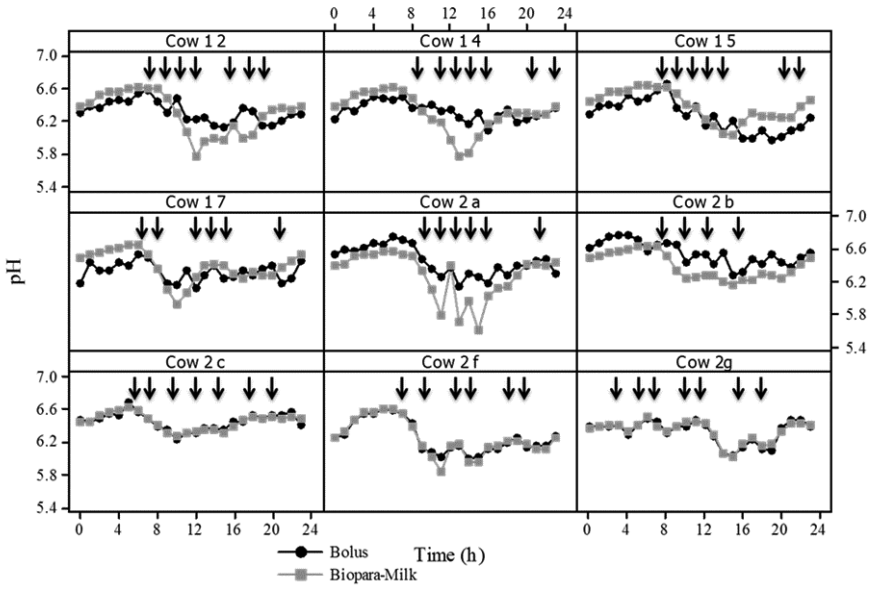

Figure 2. Circadian pH dynamics obtained with Biopara-Milk and by intra-ruminal boluses per cow. The arrows represent feeding patterns (each individual meal).

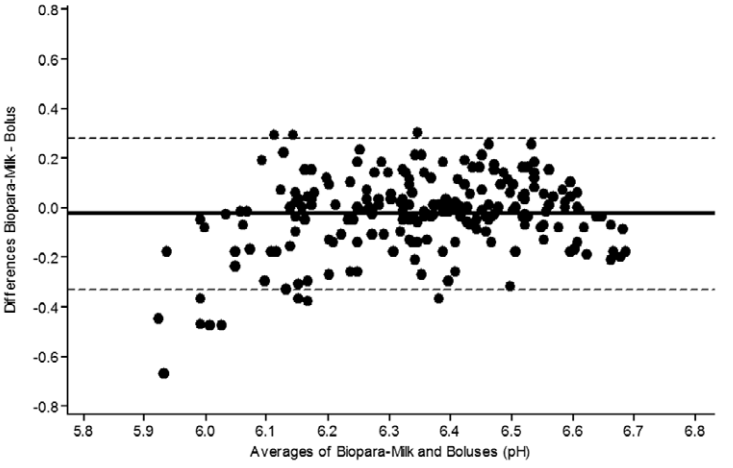

Figure 3. The limits of agreement method with multiple observations per individual. The plot shows pH values per hour per cow obtained with the pH boluses and with Biopara-Milk. The lines represent the main difference between the two methods (central solid line, -0.02) and the limits of agreement higher (upper broken line 0.29) and lower (lower broken line -0.33).

Figure 4 shows the pooled data for the means of pH per hour from all the cows obtained with both Biopara-Milk and the rumen boluses. Biopara-Milk rumen pH predictions were highly correlated to those recorded with the rumen boluses (r=0.93, P<0.005, CCC=0.85, n=24).

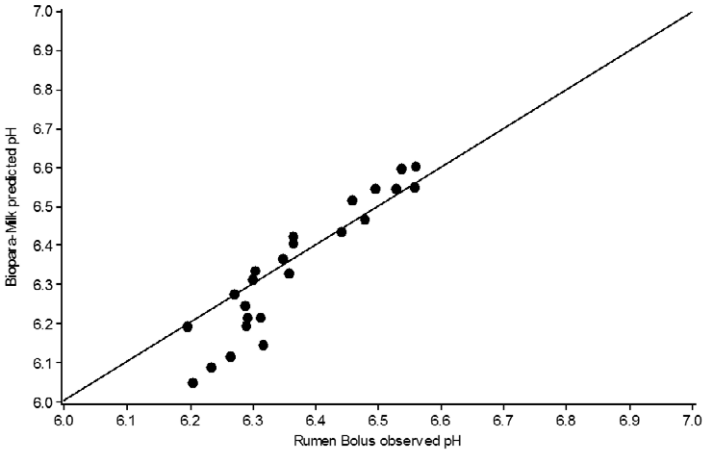

Figure 4. Pooled data for rumen pH per hour for all cows obtained with Biopara-Milk and the rumen pH boluses.

Conclusions

Modelling allows the simulation of several aspects of dairy cow physiology, and such simulations can be used to evaluate the effect that feeding regimes have on ruminant physiology and production. Evaluation of the results of such simulation exercises is of benefit as a means of testing assumptions regarding rumen physiology and environment. Measurement of rumen pH dynamics from rumen boluses can be used to evaluate the suitability of such models. Predicting rumen pH dynamics involves many assumptions: firstly, the rumen bicarbonate levels depend on salivary input (variation in saliva production rates between resting, eating and ruminating), passage rate, absorption of VFA and level of bicarbonate in the feed; secondly, the production of acid depends on the diet and its degradation, microbial metabolism, passage and absorption, and lastly, the calculation of resultant bicarbonate levels per hour. Given an accurate description of the animals and the feed consumed, Biopara-Milk is capable of accurately predicting pH dynamics in dairy cows. The simulation exercise has shown the capabilities of Biopara-Milk to predict pH dynamics in dairy cows. Future work will explore the use of Biopara-Milk as a diagnostic tool for rumen pH related diseases such as sub-acute rumen acidosis.

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